VOLUME XCI NUMBER 2

THE

BOTANICAL GAZETTE

April 1931

EFFECTS OF VARIATION IN DAY-LENGTH AND CLIPPING OF PLANTS ON NODULE DEVELOPMENT AND GROWTH OF SOY BEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 412

SCOTT V. EATON

(WITH FIVE FIGURES)

Introduction

The importance of nitrogen fixation by legumes is universally recognized, and need not be emphasized here. In the case of most of the elements essential for the growth of plants, rocks constitute the original source. This, however, is not true of nitrogen, for the original unweathered rocks of the earth's crust are devoid of it (**5**). The ultimate source of the soil nitrogen is the atmosphere; here there is an inexhaustible supply. Over each acre of the earth's surface there are about 69,000,000 pounds of nitrogen (**17**), but, so far as we know, the ability to fix this nitrogen is possessed only by certain microorganisms, for example, certain free-living bacteria, notably *Azotobacter*, and the symbiotic or nodule-forming bacteria, various strains of *Bacillus radicicola*. Soil nitrogen, therefore, which is essential for the growth of all plants, has its ultimate source in this nitrogen-fixation process. There have been a great number of tests to determine how much nitrogen various legumes fix. While results vary with different legumes and different conditions, most of the data indicate that 100–200 pounds of nitrogen are fixed by an acre of a leguminous crop (**8**).

Because of the importance of nitrogen fixation by legumes, there has naturally accumulated a voluminous literature on the effects of various factors on nodule formation. No effort is made to review this literature. It is well summarized by various investigators (**8, 18**). Among the great number of papers on the factors affecting nodule formation, however, there are comparatively few which are concerned with those factors affecting the amount of carbohydrate manufactured by the plant in the process of photosynthesis.

LEONARD (**10**) found that when the amount of photosynthetic carbohydrate was decreased by growing the plants during the short days of winter, by clipping them, or by growing them in an atmosphere low in carbon dioxide, there was poorer nodule development than when photosynthesis was not decreased in any of these ways. ROSENFELS (**16**) grew soy beans in both poor and rich soil, exposing part of the plants of each series to a short day and part to a long day. He found that the short-day plants in each type of soil had more nodules than the corresponding long-day plants, and that the short-day, poor-soil plants had more nodules than the short-day, rich-soil plants. None of the long-day rich-soil plants developed nodules, and only 8.6 per cent of the long-day poor-soil plants. Nodule development seemed to be favored by a short day and by poor soil.[1]

The purpose of this investigation was to secure additional information on the effects on nodule development of varying the amounts of carbohydrate manufactured by the plant. It is known that root development is favored by a large supply of available carbohydrates in proportion to the amount of nitrogen present (**15**). It was thought that if photosynthesis should be decreased by shortening the daily exposure to light, or by clipping the plants, there might be poorer root and nodule development on account of a lack of carbohydrates in proportion to the nitrogen supply. Nitrogen fixation has usually been considered an endothermic process. As pointed out by LEON-

[1] Since this paper was written, an article by WELTON and MORRIS has appeared (WELTON, F. A., and MORRIS, V. H., Effect of fertility on the carbohydrate-nitrogen relation in the soy bean. Plant Physiol. **5**:607–612. 1930). They found, as did ROSENFELS, that soy bean plants grown in poor soil developed more nodules than when grown in rich soil, and also had a higher percentage of carbohydrates but about the same percentage of nitrogen.

ARD (10), it is usually considered that the bacteria make use of carbohydrates manufactured by the plant in the process of photosynthesis. It was thought that by reducing photosynthesis in the ways just described a point might be reached where nodule development would fail, at least partly, on account of there not being enough carbohydrate to serve as energy and building material in this endothermic process.

Professor CHARLES A. SHULL suggested the problem to the writer. Two of Dr. SHULL'S students, Dr. A. F. BARSS and Miss NAOMI MULLENDORE, studied as a class problem the effects on the growth of Golden Wax beans and of peas of brief exposures to light. The exposures varied from 1 minute to 4 hours per day. In the case of the beans there were no nodules observed on the roots except in the case of the plants exposed to light 4 hours per day, and here only one nodule of any size was found. It was thought worth while to try longer exposures. Most of the experimental work was done in the spring of 1929, but at the suggestion of Professor E. J. KRAUS, part of the work was repeated in the fall of 1929.

Methods

In all experiments plants of *Soja major* (L.) Piper were used. There were three distinct experiments. Experiments I and II were run in triplicate; experiment III in quadruplicate.

EXPERIMENT I.—This experiment consisted of the exposure of soy bean plants to daylight for periods varying in length. The following exposures were made, the figures giving the number of hours' exposure each day and the period of the day during which the exposure was made: 2 hours, 9:50 A.M.–12:00 noon; 3 hours, 9:50 A.M.–1:00 P.M.; 4 hours, 9:50 A.M.–2:00 P.M.; 5 hours, 9:00 A.M.–2:00 P.M.; 6 hours, 9:00 A.M.–3:00 P.M.; 8 hours, 8:00 A.M.–4:00 P.M.; 10 hours, 6:00 A.M.–4:00 P.M.; 12 hours, 6:00 A.M.–6:00 P.M.; full day-length, about 13.5 hours. This plan of exposure was followed except during the last few days of the experiments, when the change of time to the daylight-saving system necessitated a few slight changes as to the exposure periods. It will be noticed that in a few cases the length of the exposures does not correspond exactly with the number of hours indicated. The changes were made necessary

on account of classes, but, for convenience, throughout the paper the number of hours' exposure to light is given in whole numbers. When the plants were not being exposed to the light they were kept in a dark box in the same room in which the light exposures were made.

EXPERIMENT II.—The plants of this experiment were clipped with different degrees of severity. Those of one series were not clipped at all. These were the same plants as those of the full day-length series of experiment I. From the plants of a second group the cotyledons and first true leaves were removed. The plants of a third group were kept clipped to one leaf.

EXPERIMENT III.—Experiments I and II were tried in the spring of 1929; experiment III, in the fall of 1929. In the case of the latter experiment, one group of plants was exposed to 7 hours of natural illumination per day, the period of illumination being 8:00 A.M.–3:00 P.M. The plants of a second group were exposed to natural daylight until 4:00 P.M., at which time two 1000 watt bulbs, placed about 2 feet above the plants, were turned on. These were left on until 10:00 P.M., thus giving a day-length of about 16.3 hours.

The seeds used in all the experiments were of the variety Wisconsin Black, and were secured through the kindness of Dr. L. F. GRABER of the Department of Agronomy of the University of Wisconsin. Before planting the seeds they were inoculated with an inoculum kindly supplied by Dr. I. L. BALDWIN of the Department of Agricultural Bacteriology of the University of Wisconsin. The seeds were planted in no. 3 pure quartz sand in 2-gallon glazed earthenware crocks. Twenty pounds of sand were weighed out for each crock. In the bottom of each crock was a hole to allow for drainage. In order to get uniform plants, seeds of as nearly uniform size as possible were selected, although the individual seeds were not weighed. An excess of seeds was planted in each pot, and about 2 weeks after they had sprouted they were thinned to 6 uniform plants to the pot. In the case of a few pots, 6 uniform plants were not obtainable, so a smaller number was left. It is believed that by this plan moderately comparable material in the different series was obtained.

The nutrient solution used was a modified form of that recom-

mended by BRYAN (2). The composition of this solution, as used by BRYAN, was as follows:

Solution A: $CaCl_2$, 50 gm.
$Ca(NO_3)_2$, 50 gm.
Distilled water, 500 cc.

Solution B: $MgSO_4$, 50 gm.
Distilled water, 500 cc.

Solution C: K_2HPO_4, 50 gm.
Distilled water, 500 cc.

Solution D: $FeCl_3$, 2.5 gm.
Distilled water, 300 cc.

Ten cc. of each of solutions A, B, C, and 10 drops of solution D were added to 4 liters of distilled water. This solution was modified by reducing the nitrate content of solution A to such a point that when diluted for use the nutrient solution contained 3 mg. of nitrate nitrogen per 100 cc. FRED and co-workers (6) found that if there was present a larger amount of nitrate nitrogen than 5 mg. per 100 cc. of nutrient solution, nodule development was partly inhibited in the case of vetch. The plants were watered daily. For the most part the watering was done with the nutrient solution, but on 2 days each week distilled water was added instead of the solution. The nutrient solution and the water were sprinkled on to the surface of the sand with a small sprinkling can. Twice each week enough of the nutrient solution was added so that it ran out through the holes in the bottom of the crocks. It was thought that this manner of adding the solution facilitated aeration, and also helped to keep the solution at a constant concentration. While the pots were not weighed, an attempt was made to keep the sand in each pot equally moist.

It was not possible to control accurately the temperature and humidity in the greenhouse, and there were considerable variations in these conditions from day to day. There may have been also, especially on bright warm days, some differences in these conditions inside the box and outside. Both in regard to temperature and humidity in the greenhouse, and differences in these conditions inside and outside the box, conditions were much more constant in the autumn than in the spring. Measurements showed that in the autumn the temperature and the humidity did not change much from day to day in the greenhouse, and that the differences inside and outside the box were small. Although these conditions were not as constant as was desired, the rather definite and regular response

of the plants, especially as regards size and color, to variations in the day-length and in the severity of clipping certainly indicated that differences in the treatment and not in temperature and humidity accounted for the results obtained.

The seeds for experiments I and II were planted March 29. The first plants were observed coming up on April 5. The plants were harvested on May 3, at which time there were a few blossoms on the plants exposed to full day-length. The seeds for experiment III were planted September 29, and the first plants were observed coming through the sand on October 7. Harvesting was done on November 6, at which time the short-day plants were in bloom and a few small pods had formed. No buds were observed on the long-day plants. Late in the evening of the day before the plants were harvested, all the plants of each experiment were put in the dark box and left until harvested next day.

SAMPLING.—The sand was carefully shaken from around the roots by jarring the crock on a board; the roots were immersed in a 20 per cent salt solution to remove the sand, and rinsed in distilled water. The plants were then wrapped in several thicknesses of newspaper and taken at once to the laboratory. Each series was harvested separately. At the laboratory the tops and roots were separated and the nodules picked from the roots. The nodules were weighed, a little 95 per cent alcohol was added, and they were later dried to constant weight in the vacuum oven at a temperature of about 80°C. The tops and roots were sampled separately. They were ground twice through a Russwin mill. About 0.25 gm. of $CaCO_3$ was added to each Erlenmeyer flask to neutralize any acids present in the tissue. The samples were weighed out, enough hot 95 per cent alcohol added to make the final percentage at least 80 per cent, and the samples heated on the steam-bath for 1 hour, small funnels being placed in the mouths of the Erlenmeyer flasks to reduce the evaporation of the alcohol. Any alcohol evaporating during the heating was replaced. The samples were stoppered tightly and stored.

METHODS OF ANALYSIS.—In the case of experiments I and II, the moisture content of each sample was determined by subtracting the sum of the dry weight of the residue left after the extraction of the sugar, and the dry weight of the total solids of the sugar extract

from the wet weight of the sample. The moisture content of the plants of experiment III was determined by taking duplicate samples of the ground pulp in tared watch glasses, which had ground edges and were held together by means of a clamp, and drying to constant weight in the vacuum oven.

The sugar extract was obtained by extracting the pulp on the steam-bath in 80 per cent alcohol for a total period of not less than 4 hours, the extract being poured off three times during this period. After the extraction, the pulp was brought on to the filter and washed with hot 80 per cent alcohol. All the extracts from each sample were made up to the mark in a volumetric flask. Tests showed that this method gave complete extraction of the sugar.

The sugar extract was freed of alcohol by evaporation on the steam-bath, cleared with neutral lead acetate, and deleaded with potassium oxalate. The reducing sugars were determined in the extract thus obtained. For the determination of the total sugars, 5 cc. of HCl was added to 50 cc. of the prepared extract; the solution was heated on the metal part of the steam-bath for 1 hour, and then set aside for 24–48 hours. The extract was neutralized with sodium carbonate and the reduction made.

The acid-hydrolyzable material was determined in the residue left after the extraction of the sugar. This residue was ground to a fine state and dried to constant weight in the vacuum oven. Hydrolysis was effected by adding to each sample 200 cc. of water and enough HCl to make a 2.5 per cent solution, and boiling for 3 hours under a reflux condenser. The sugar extract was cleared and deleaded as before and the sugar determined in this prepared extract.

In all cases the sugar of the extracts was determined by the gravimetric method, the sugar equivalent of the cuprous oxide being ascertained by reference to the Munson and Walker tables. The total sugars and the reducing sugars were calculated as invert sugar; the acid-hydrolyzable material as glucose. The percentage of sucrose was found by subtracting the percentage of the reducing sugars from the percentage of the total sugars and multiplying the result by 0.95.

Total nitrogen was determined separately in the sugar extract and the dry residue by the Kjedahl method modified to include the

nitrogen of nitrates. The method used was essentially that of RANKER (14). The samples of the sugar extract were evaporated to dryness on the steam-bath before making the determination.

Data

EXPERIMENT I: EFFECTS OF VARYING DAY-LENGTH IN SPRING

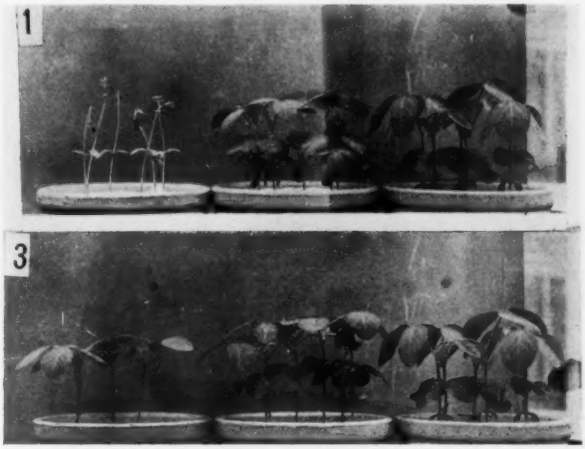
SIZE, COLOR, AND BUD DEVELOPMENT.—On April 29, 4 days before harvesting, observations were made on size, color, and bud development of all the plants. The 2-hour plants died about 3 weeks after coming through the sand; so not many data were obtained from this series. The plants of the other series showed a gradual decrease in size with decrease in the length of daily exposure to light. The decrease in size had to do not so much with their height, although there was some decrease here, as with size of leaflets, thickness of stems, and general thriftiness. The plants of the shorter exposures, especially the 3-hour plants, had a rather sickly appearance. There were brown spots on the leaves. Spots of the same kind were observed on the leaves of the 2-hour plants, before these died, and at this time they were more numerous on the plants of this series than on the 3-hour plants. Fig. 1 gives some idea of the appearance of the plants of the 2-hour, 8-hour, and full day-length exposures. This picture was taken on April 25. The 2-hour plants are practically dead; the 8-hour plants are somewhat thrifty looking although definitely smaller than those of the full day-length series.

The plants of the full day-length, 12-hour, and 10-hour series showed no marked differences in color, although the 10-hour plants were somewhat less green than the full day-length and the 12-hour plants. But from the 10-hour series to the 3-hour inclusive, there was a gradual decrease in degree of greenness.

Well developed buds were observed on the plants of the 8-hour and longer exposures. The plants of the 6-hour, 5-hour, and 4-hour exposures showed definite buds, although not so well developed as in the case of the plants of the longer exposures. No buds were observed on the 3-hour plants.

The well known effect of etiolation, one characteristic of which is lengthening of the internode, was evident. On April 20 the height of each plant in each series was determined. The average height (in

cm.) of the plants of each series was as follows: full day-length, 8.2; 12-hour, 7.7; 10-hour, 8.0; 8-hour, 7.9; 6-hour, 9.7; 5-hour, 9.9; 4-hour, 9.4; 3-hour, 12.3; 2-hour, 15.0. The plants of the 6-hour and shorter exposures clearly show the effect, and it is especially marked in the case of the 3-hour and the 2-hour plants. On April 29 the plants of the full day-length and the 3-hour series were again measured. (The 2-hour plants were dead at this time.) The plants of the former series averaged 13.8 cm. in height; of the latter, 12.8 cm.



FIGS. 1, 3.—Fig. 1, effects of exposing soy bean plants to different day-lengths in spring: left, exposed to sunlight 2 hours per day; middle, 8 hours; right, full day-length (about 13.5 hours). Fig. 3, effects of clipping: left, clipped severely, only one fully developed leaf being left; middle, only cotyledons and first true leaves removed; right, unclipped.

Owing to their greater food supply, the plants exposed to full day-length increased much more in height than the plants exposed to only 3 hours of daylight each day, and more than overtook the latter, which increased very little in height.

WEIGHTS AND RESULTS OF CHEMICAL ANALYSES.—Table I shows that as the day-length decreases there is a decrease in the dry weights of the nodules, and correlated with this decrease is a decrease in the dry weights of the plants and in the amounts of total

sugars, acid-hydrolyzable material, total carbohydrates, and total nitrogen. The table also gives the number of nodules for the plants of each series. As is probably to be expected, these data are not so closely correlated with the weights of the plants and the amounts of their chemical constituents as are the weights of the nodules. Fig. 2 gives the data in a graphical way. It will be noted that all the curves flatten out in the parts having to do with the shorter exposures. But, beginning with the 5-hour or 6-hour exposure, all

TABLE I

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES*	DRY WEIGHT	TOTAL SUGARS	ACID HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN	DRY WEIGHT OF NODULES	NO. OF NODULES
F	10.682	0.105	1.670	1.775	0.380	0.923	176.6
12	8.855	0.104	1.414	1.518	0.273	0.709	225.0
10†	1.884	0.020	0.346	0.366	0.028	0.550	146.6
8	5.991	0.062	0.937	0.999	0.231	0.258	107.1
6	4.510	0.054	0.656	0.710	0.190	0.040	48.2
5	2.931	0.021	0.476	0.497	0.145	0.012	24.6
4	2.097	0.006	0.319	0.325	0.118	0.002	12.0
3	1.795	0.000	0.259	0.259	0.089	0.001	5.0

* In this and the following tables, the letters and figures of the "Series" column indicate: F = full day-length (about 13.5 hours between sunrise and sunset); FE = full day-length plus electric light (about 16.3 hours' total exposure); T = tops; R = roots; U = unclipped; CL = clipped lightly; CS = clipped severely; figures are the no. of hours' exposure to light per day.

† Data of this series are for roots only.

the curves rise rather sharply and more or less parallel with one another, except the curve for the total nitrogen, which remains flatter than the others and is not parallel with them. No data are given for the 2-hour series, which died early. It should be stated, however, that the roots of this series were examined, and 6 very small nodules observed.

Table II gives the data for the tops and roots separately. It is to be noted that, as in the case of the entire plant (table I), the data for the tops and the roots are correlated with the length of exposure, although the correlation is closer in the case of the tops than in the case of the roots. The weights of the nodules (table I), therefore, which are correlated with the length of the exposure, the dry weights, and the amounts of the chemical constituents of the entire plant, are more closely correlated with the dry weights and the total

amounts of the chemical constituents of the tops than with these data for the roots.

Tables III and IV give data for the percentage chemical composition of the plants. Dry weights of nodules (table I) are correlated, although not in an exact way, with the percentages of acid-hydrolyzable

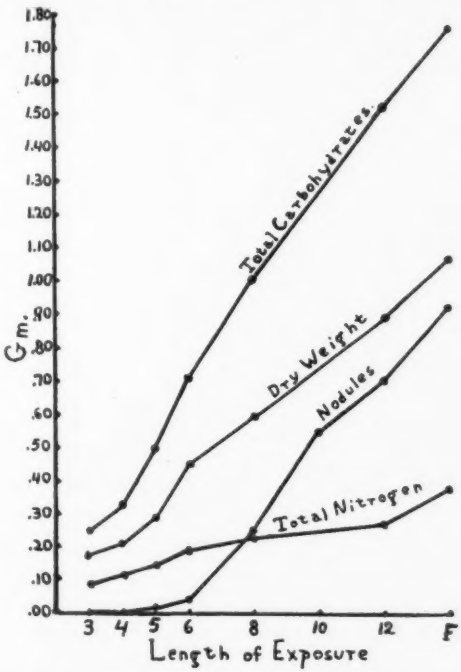


FIG. 2.—Effects of exposing soy bean plants to different day-lengths in spring; absolute gm. per 10 plants, tops and roots included: total carbohydrates, dry weight of plants (divided by 10), dry weight of nodules, and total nitrogen; figures on abscissae are number of hours' exposure to sunlight per day (F=full day-length, about 13.5 hours).

material and dry weights of the tops, but not with the corresponding percentages of the roots. Both dry weights of nodules and percentages of acid-hydrolyzable material in the tops decrease with decrease in the length of exposure to light. Percentages of dry weight, however, are even more closely correlated with weights of nodules than

are percentages of acid-hydrolyzable material. There is no correlation between percentages of total sugars in either tops or roots and weights of nodules.

TABLE II

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATELY

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
FT	8.533	0.086	1.297	1.383	0.339
12T	6.509	0.076	0.983	1.059	0.233
10T	.....	.....	.....	.....	.....
8T	4.659	0.055	0.698	0.753	0.198
6T	3.214	0.040	0.449	0.489	0.164
5T	2.174	0.014	0.304	0.318	0.129
4T	1.513	0.006	0.211	0.217	0.098
3T	1.253	0.000	0.157	0.157	0.083
FR	2.149	0.019	0.373	0.392	0.041
12R	2.346	0.028	0.431	0.459	0.040
10R	1.884	0.020	0.346	0.366	0.028
8R	1.332	0.007	0.239	0.246	0.032
6R	1.296	0.014	0.207	0.221	0.026
5R	0.757	0.007	0.172	0.179	0.016
4R	0.584	0.000	0.108	0.108	0.020
3R	0.542	0.000	0.102	0.102	0.006

TABLE III

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT DRY WEIGHT AND MOISTURE

SERIES	MOISTURE*	DRY WEIGHT*	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL NITROGEN
FT	83.862	16.138	1.022	15.200	3.976
12T	85.873	14.127	1.179	15.200	3.588
10T	.....	.....	.....	.....	.....
8T	86.525	13.475	1.196	15.000	4.265
6T	86.203	13.797	1.268	14.000	5.124
5T	89.537	10.463	0.673	14.000	5.954
4T	90.740	9.260	0.442	14.000	6.493
3T	91.155	8.845	0.000	12.560	6.651
FR	90.634	9.366	0.883	17.400	1.947
12R	89.443	10.557	1.212	18.400	1.712
10R	90.678	9.322	1.072	18.400	1.537
8R	90.908	9.092	0.579	18.000	2.457
6R	78.816	21.184	1.156	16.000	2.044
5R	84.132	15.868	1.050	22.800	2.198
4R	87.682	12.318	0.000	18.500	3.523
3R	84.878	15.122	0.000	18.933	1.195

* Moisture and dry weight percentages are on basis of wet weight of sample.

Percentages of moisture in the tops increase with decrease in length of exposure to light, as is probably to be expected. This is not true of the roots. It would seem that an error must account for the very low moisture content of the roots of the 6-hour exposure, which of course affects the other data for this series. The percentage nitrogen content of the tops increases with decreasing day-length. The data for the percentage nitrogen content of the roots show considerable irregularity. Sucrose was determined in only three of the series. Table IV shows that most of the total sugars of these series were made up of reducing sugars. On the average,

TABLE IV

PERCENTAGE OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
FT.....	1.022	0.766	0.243
12T.....	1.179	0.955	0.212
8T.....	1.196	0.892	0.288

the reducing sugars made up about three-fourths of the total and sucrose one-fourth.

The top-root ratios were determined by dividing the dry weights of the roots of 10 plants into the dry weights of the tops of 10 plants. The ratios for the different series are: Ft, 3.970; 12T, 2.774; 10T, ——; 8T, 3.497; 6T, 2.479; 5T, 2.871; 4T, 2.590; 3T, 2.311. It is to be noted that the plants of the longest exposure had the largest ratio and those of the shortest exposure the smallest, but that there is no definite gradation between these two extremes.

EXPERIMENT II: EFFECTS OF CLIPPING

Fig. 3 shows the appearance of the plants. Tables V-VIII give the data for this experiment. Table V shows strikingly the effects of clipping on nodule development. The more severely the plants were clipped the less was the weight of the nodules. The table also shows that the weights of nodules are correlated with the dry weights of the plants and with the amounts of total sugars, acid-hydrolyzable material, and total carbohydrates.

As in the case of experiment I, it is to be noted that the number of the nodules is not as closely correlated with the dry weights and

TABLE V

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES*	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN	DRY WEIGHT OF NODULES	NO. OF NODULES
U	10.682	0.104	1.670	1.774	0.380	0.923	176.6
CL	6.828	0.054	1.061	1.115	0.241	0.645	181.2
CS	3.388	0.031	0.550	0.581	0.102	0.242	112.3

* U = unclipped; CL = clipped lightly; CS = clipped severely.

TABLE VI

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATE

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
UT	8.533	0.086	1.297	1.383	0.339
CLT	4.862	0.036	0.719	0.755	0.210
CST	2.122	0.020	0.318	0.338	0.080
UR	2.149	0.018	0.373	0.391	0.041
CLR	1.966	0.018	0.342	0.360	0.031
CSR	1.266	0.011	0.232	0.243	0.022

TABLE VII

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT DRY WEIGHT AND MOISTURE

SERIES	MOISTURE*	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL HYDROGEN
UT	83.862	16.138	1.022	15.200	3.976
CLT	85.910	14.090	0.756	14.800	4.338
CST	86.443	13.557	0.973	15.000	3.793
UR	90.634	9.366	0.883	17.400	1.947
CLR	90.352	9.648	0.953	17.400	1.592
CSR	91.461	8.539	0.938	18.400	1.788

* Moisture and dry weight percentages are on the basis of wet weight of sample.

the amounts of the chemical constituents of the plants as are the weights of the nodules. Fig. 4 records the data in the form of curves. These are almost straight lines, and also approximately parallel with

one another, except the curve for the total nitrogen, which, as in the case of experiment I, is somewhat flatter than the other curves.

Table VI shows that the dry weights of the nodules (table V) are correlated in a general way with the dry weights and the amounts of the chemical constituents of tops and roots considered separately, although more closely correlated with these data for the tops than for the roots. It should be stated that the clippings were not saved, so that the data for the tops, in the case of the clipped series, are for the part left at the time of harvest.

When the percentage composition of the plants is considered (table VII), however, it is seen that the only correlation is between weights of nodules and percentages of dry weights of the tops, there being no correlation between weights of nodules and percentages of dry weights of the roots or percentages of sugar or acid-hydrolyzable material in either tops or roots. The percentage of moisture of the tops increases directly with the severity of the clipping. This is probably to be expected, since the more severely the plants are clipped the more transpiring surface is removed. The moisture percentages of the roots bear no direct relation to the severity of the clipping. The percentages of total nitrogen do not vary directly with the severity of the

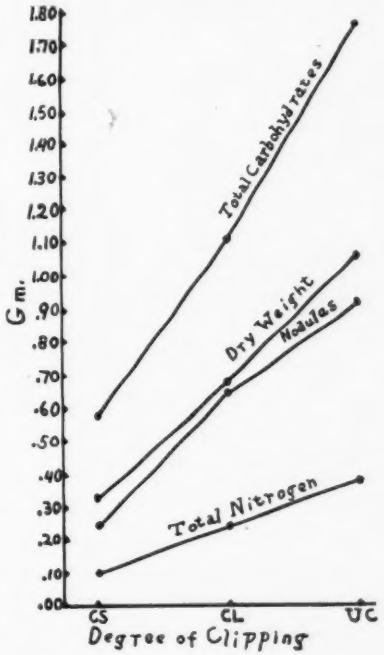


FIG. 4.—Effects of clipping; absolute gm. per 10 plants, tops and roots included: total carbohydrate, dry weight of plant (divided by 10), dry weight of nodules, total nitrogen. CS = clipped severely, to one fully developed leaf; CL = clipped lightly, only cotyledons and first true leaves removed; UC = unclipped.

clipping in either tops or roots. Table VIII shows that sucrose makes up a greater percentage of the total sugars in the case of the plants which were clipped lightly than in the case of the plants which were not clipped. This may be due at least partly to the fact that the clippings were not saved.

TABLE VIII

PERCENTAGES OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
UT.....	1.022	0.766	0.243
CLT.....	0.756	0.499	0.244

The top-root ratios were determined. The ratios for the three series are: FT, 3.970; CLT, 2.473; CST, 1.676. Since the clippings were not saved these ratios are probably not significant.

EXPERIMENT III: EFFECTS OF VARIATION IN DAY-LENGTH IN FALL; NATURAL DAYLIGHT SUPPLEMENTED WITH ELECTRIC LIGHT

Fig. 5 gives some idea of the differences in appearance of the long- and the short-day plants. The picture was taken a few days before the plants were harvested. The long-day plants were much taller than the short-day plants and were twining about the supports, while the short-day plants were not twining. On November 2, 4 days before the plants were harvested, the longest plant in each pot was measured and observations made of all the plants as to color, occurrence of hairs, and bud development. The average height of the long plants in the long-day series was 43.7 cm.; in the short-day series, 22.5 cm. The long-day plants were much darker green than the short-day plants and showed a much greater development of hairs. There were a number of buds on each of the short-day plants and a few had opened. No buds were observed on the long-day plants. When the plants were harvested, 4 days later, a few small pods were observed on the short-day plants.

The weights of the plants and the results of the chemical analyses are found in tables IX-XII inclusive. By reference to table IX, it

is seen that the weight of the nodules of the long-day plants is much greater than that of the short-day plants, and that correlated with this greater weight of nodules is a greater dry weight and greater amount of total sugars, acid-hydrolyzable material, total carbohydrates, and total nitrogen in the case of the long-day plants as compared with the short-day plants. The weights of the nodules are on

FIG. 5.—Effects of exposing soy bean plants to different day-lengths in autumn: left, exposed to sunlight 7 hours per day; right, exposed to sunlight from sunrise to 4:00 P.M. and to electric light from then to 10:00 P.M., giving total exposure of about 16.3 hours.

the wet weight basis. Since the nodules probably have about the same percentage of moisture as the rest of the plant, however, this fact does not greatly vitiate the comparison of weights of nodules and dry weights of plants. Table X shows that this same comparison holds for the tops and roots considered separately, although more closely for the tops than for the roots.

Table XI gives the percentage composition of the plants; table XII gives the percentages of the sugar fractions. Weights of nodules

(table IX) are correlated in a general way with percentages of acid-hydrolyzable material of tops and roots, although the correlation is

TABLE IX

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN	WET WEIGHT OF NODULES	NO. OF NODULES
FE	13.477	0.375	2.708	3.083	0.478	4.136	167.3
7	4.917	0.107	0.790	0.897	0.208	0.663	86.3

TABLE X

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATE

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
FET	11.409	0.335	2.384	2.719	0.441
7T	3.889	0.080	0.645	0.725	0.196
FER	2.068	0.040	0.324	0.364	0.037
7R	1.028	0.027	0.145	0.172	0.012

TABLE XI

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT DRY WEIGHT AND MOISTURE

SERIES	MOISTURE	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL NITROGEN
FET	84.958	15.042	2.937	20.900	3.874
7T	87.197	12.803	2.077	16.600	5.044
FER	86.641	13.359	1.944	15.700	1.824
7R	86.814	13.186	2.650	14.200	1.203

TABLE XII

PERCENTAGE OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
FET	2.937	2.325	0.581
7T	2.077	1.288	0.749

closer for the tops than for the roots. The greater weight of nodules of the long-day plants is also correlated with a greater percentage

of total sugars of the tops of these plants. This comparison, however, does not hold for the roots. Weights of nodules are correlated with percentages of dry weights of tops and roots, although the correlation is closer for the tops than for the roots.

Both the tops and the roots of the short-day plants have a higher percentage of moisture than the long-day plants, although the percentage is not much greater in the case of the roots. The percentage of total nitrogen is higher in the case of the short-day tops, but the reverse holds for the roots. The top-root ratios were determined. The ratio for the long-day plants was 5.516 and for the short-day plants, 3.783. The ratio of the long-day plants was the highest obtained in any of the experiments, while the ratio of the short-day plants is of the same order as the ratios of experiment I.

Discussion

Effects on nodule development of variations in factors affecting amounts of carbohydrate manufactured

Two aspects of the relation of the chemical composition of the plant to its development should be mentioned in this connection. One is the relation of carbohydrates and nitrogen to the root development; a second is the need of the legume for carbohydrates in the process of nitrogen fixation.

Various investigators have found that a large carbohydrate supply in proportion to the amount of nitrogen present favored root development, while the reverse situation favored shoot development. For example Reid (**15**), working with tomato cuttings, found that there was better root development when the cuttings contained a large supply of readily available carbohydrates in proportion to the amount of nitrogen present, but that there was poor root development when the cuttings contained a low carbohydrate supply in proportion to the amount of nitrogen present. It might be expected that with a decrease in day-length, or because of clipping, the percentage of carbohydrates in the plant (roots, tops, or both) would be less, and that this low carbohydrate supply in proportion to the nitrogen would result in decreased root development, and also, since the nodules may be considered a part of the root system, in decreased nodule development.

A second aspect of the question is the need of carbohydrates for the process of nitrogen fixation. This process has usually been considered an endothermic one, and it is thought that complex organic compounds, perhaps protein in nature, are synthesized. It might perhaps be expected, therefore, that when carbohydrate manufacture is reduced there would be poorer nodule development, owing to there not being sufficient carbohydrates to serve as energy and building material for this endothermic process. LEONARD (**10**) found that when photosynthesis by soy bean plants was decreased, owing to insufficient light, CO_2, or chlorophyll, there was poorer nodule development than in the case of plants suffering no reduction in the amount of carbohydrates manufactured. This was explained by assuming that there was not sufficient carbohydrates for the activities of the nodule-forming bacteria after the needs of the plant were supplied. ROSENFELS (**16**) found that short-day plants grown in poor soil developed many more nodules than long-day plants grown in the same type of soil, and that correlated with this better nodule development was a higher starch content. It was stated that this is to be expected because of the need of carbohydrates for the synthesis of the higher organic complexes. However, this correlation between nodule development and starch content was not found in the case of short-day and long-day, rich-soil plants.

In view of these two considerations, the relation of carbohydrates and nitrogen to root development, and the need of carbohydrates for nitrogen fixation, it becomes of interest to inquire as to what correlation was found between the chemical composition and the growth and nodule development of the plant as a result of variations in day-length and clipping. As shown by tables III and XI, the main effect of variations in day-length on the chemical composition of the plant was that as the day-length decreased the percentage of acid-hydrolyzable material of the tops decreased and the percentage of nitrogen increased. In the case of experiment III, table XI (but not of experiment I) this was also true, although to a lesser extent, of the roots; also the percentage of total sugars of the tops of the short-day plants of this experiment was less than that of the long-day plants. In other words, the short-day plants of experiments I and III had a smaller percentage of carbohydrates and a

higher percentage of nitrogen than the corresponding long-day plants. This is the situation usually prevailing in the case of more vegetative, less fruitful plants, which also have usually a smaller developments of roots in proportion to the tops. But the short-day plants of each experiment were less vegetative, and had a greater development of roots in proportion to the tops than the long-day plants. Also the short-day plants in each case were fruitful, although in the case of experiment I the long-day plants blossomed first. With a decrease in day-length there was poorer nodule but better root development. As for the effects of clipping, although the weights of nodules varied directly with its severity, table VII shows that the percentages of the various chemical constituents did not vary in any regular manner with the degree of clipping. This may be due at least partly to the fact that the clippings were not saved. The results do not seem to show a very close correlation between the carbohydrate-nitrogen relationship of the plant and growth and nodule development.

When the data are considered from the standpoint of the need of carbohydrates for the process of nitrogen fixation, percentages of carbohydrates of the plant seem to be more closely correlated with nodule development than was noted for the carbohydrate-nitrogen relationship of the plant. The long-day plants of experiments I and III had better nodule development than the plants exposed to sunlight a shorter length of time per day (tables I and IX). Also, as noted in the preceding paragraph, these long-day plants were higher in carbohydrates, especially acid-hydrolyzable material. But the correlation is far from an exact one. In the case of experiment I both weight of nodules and percentage of acid-hydrolyzable material decreased with decrease in the length of exposure to light, but tables I and III show cases of decided decrease in weights of nodules without any decrease in the percentages of acid-hydrolyzable material. Yet one would seem justified to conclude that one reason for the decrease in nodule development as the day-length is shortened is the decreasing supply of carbohydrates for the use of the nodule-forming bacteria in the nitrogen fixation process. In the case of experiment II (effects of clipping), although the weight of nodules varied directly with the severity of the clipping, table VII shows that the per-

centages of carbohydrates varied in no regular way with this severity. This may have been due to the fact that the clippings were not saved, as previously mentioned. It must be kept in mind, however, that certain recent work minimizes somewhat the need of carbohydrates for the nitrogen fixation process. It is known that the synthesis of ammonia from the elements composing it is an exothermic process (5), and, while the initial compound formed in the fixation of nitrogen by the nodule-forming bacteria is not known, there is evidence, according to BURK (1), that *Azotobacter* fixes the nitrogen in the form of ammonia. If proteins are the final products of the fixation process, however, it would seem that the entire process must be endothermic, since the proteins have a higher heat of combustion than the carbohydrates, which serve as part of the building material for the proteins. Yet BURK states that it is practically certain that energy is not essential for the synthesis of complex nitrogen compounds from ammonia. Of course, even if the steps from ammonia on are endothermic in character, the energy for these steps could come from the exothermic fixation of ammonia. In this case the entire process, including the synthesis of the proteins, might require little if any energy. From experimental results, CHRISTIANSEN-WENIGER (3) concluded that fixation of nitrogen by the nodule-forming bacteria is an exothermic process, and that the bacteria are able to use this energy in their life processes. This viewpoint lessens somewhat the importance of carbohydrates in the nitrogen-fixation process. However, even if they are not needed as an energy source for the fixation of nitrogen, they are needed as a carbon source for the synthesis of the complex nitrogen compounds, and any deficiency in carbohydrates would be expected for this reason to result in poorer nodule development.

Although, as just stated, nodule development is correlated at least in a general way with percentage of carbohydrates, especially acid-hydrolyzable material, a closer correlation is noted when the data are examined from another standpoint. Tables I, II, V, VI, IX, and X give on the absolute rather than the percentage basis the dry weights and the amounts of the chemical constituents of the plants. As the day-length decreases, or as the plants are clipped more severely, the weights of nodules decrease, and correlated with poorer nodule de-

velopment there is less dry weight and lesser amounts of total sugars, acid-hydrolyzable material, and total nitrogen. In other words, the nodules seem to develop as the rest of the plant develops. This is perhaps to be expected since the nodules are an integral part of the plant. This correlation is closer for the tops than for the roots (tables II, VI, X). It is also striking when the data for the entire plant are considered (tables I, V, IX, and figs. 1, 4). The figures show that the curves for the weights of nodules are more nearly parallel with those for total carbohydrates than with those for total nitrogen. So, as photosynthesis is reduced there is less growth of all parts of the plants, including the nodules. According to this viewpoint, the development of tops, roots, and nodules may be better explained by a consideration of the total amounts of the chemical constituents than of the amounts figured on a percentage basis.

This idea, that development of the plant may be more closely correlated with the chemical composition figured on an absolute rather than a relative or percentage basis, is really not in conflict, at least in the case of experiments I and II, with the viewpoint that the type of plant resulting from any treatment is determined by the chemical composition figured on the latter rather than the former basis; for in these experiments, the types of plant in the various series were similar, although there were big differences in their sizes. Bud development of the plants of the various series of experiment I was similar (except the 3-hour plants, which developed no buds), although the first buds to open were on the plants of the full day-length series. The top-root ratios of experiment I were somewhat different, but the differences were not consistent from series to series. There was a big decrease in the top-root ratios of experiment II as the severity of the clipping increased, but this is due at least in part to the fact that the clippings were not saved. If we consider the type of plant as similar in the various series of experiments I and II, therefore, the amounts of the chemical constituents figured on an absolute basis might perhaps be considered more important in explaining the differences in weights of nodules, tops, and roots, than the amounts figured on a percentage basis. The total amount of the chemical constituents present determines the amount of growth. As photosynthesis is decreased there is less growth of all the

parts of the plant, because there is less food material available. The plant is smaller, but this does not necessarily mean that there is a difference in the percentage of the chemical constituents present, at least of a magnitude and character sufficient to account for the difference in the development of the plant. According to this viewpoint the type of plant is still determined by the chemical composition figured on a percentage rather than an absolute basis, but with the type of plant in the various series similar, the chemical composition figured on an absolute basis might be regarded as more important in explaining the differences resulting from the treatments. The long- and short-day plants of experiment III are very different in type, but even in this case the amounts of the chemical constituents figured on an absolute rather than a percentage basis seem more important in explaining the development of the plant, including nodule development.

It is realized that there is need of greater fractionation of the carbohydrates and the nitrogen in any interpretation of the relation of the chemical constituents to the development of the plant. Since the work of KRAUS and KRAYBILL (9), great emphasis has rightly been placed on the importance of the carbohydrate-nitrogen relationship in the development of the plant. KRAUS and KRAYBILL regarded the nitrate-nitrogen fraction as the most important nitrogen fraction. Recent work, however, especially that of NIGHTINGALE (11), emphasizes the importance of the soluble organic nitrogen. NIGHTINGALE finds that the balance between the available carbohydrates and the soluble organic nitrogen determines the type of growth of the plant. It is unfortunate that the acid-hydrolyzable material was not separated into the hemicellulose and the starch-dextrin fractions. The functions of the hemicelluloses in the plant are not definitely known. NIGHTINGALE regards the starches and dextrins as more intimately connected with the plant's metabolism than the hemicelluloses. His work indicates that, although the hemicelluloses may be utilized by the plant as a sugar source, this does not occur until the starches and dextrins become deficient. He admits, however, that his data are insufficient for definite conclusions on this point. CLEMENTS (4), on the other hand, finds that when growing conditions are favorable, large and frequent variations in

the hemicellulose content of leaves occur, the hemicelluloses apparently serving as temporary food reserves. If the total nitrogen had been separated into the various fractions and a greater fractionation of the carbohydrates made, a greater correlation might have been found between growth and nodule development of the plant and the amounts of the chemical constituents present, figured on a percentage rather than an absolute basis, although it might still be a problem to explain the fact that nodule development was poorer in the short exposures, while root development in proportion to the tops was better.

Throughout this work the tops showed much greater response to the variations in day-length and degree of clipping than did the roots. Also the differences in the chemical composition of plants as a result of the different treatments were greater and more regular for the tops than for the roots, no matter whether figured on a percentage or an absolute basis. In one of NIGHTINGALE'S experiments it was found that long-day and short-day tomato plants had similar root systems, although the tops were very different. Also there was little difference in the relationship of the carbohydrates to the nitrogen in the roots of the long-day plants as compared with the roots of the short-day plants, although there was a decided difference in this respect in the stems of the plants of the two series. Variations in conditions, which may cause decided differences in the development of the tops of plants, may not affect the development of the roots so markedly.

EXPERIMENT III: PHOTOPERIODISM AND NODULE DEVELOPMENT

It is interesting to note that long- and short-day plants grown in the fall developed differently from the corresponding plants grown in the spring. It has been stated that when the day-length in the spring was varied the resulting plants did not vary much in type, although with decrease in day-length the resulting plants of course were smaller and less green. On the other hand, the long- and short-day plants grown in the fall were very different in type, the short-day plants being relatively non-vegetative, fruitful, and non-twining, while the long-day plants were relatively vegetative, non-fruitful, and twining (fig. 5). In regard to nodule development, however,

the plants grown in the fall were similar to those grown in the spring. In each case the long-day plants developed a much greater weight of nodules than the short-day plants (tables I and IX).

Regarding percentage composition, the long-day and the short-day plants grown in the fall were also similar to those grown in the spring. In each case the long-day plants were higher in carbohydrates, especially acid-hydrolyzable material, and lower in total nitrogen than the short-day plants (tables III and XI).

In the discussion of experiment I, it was stated that there was not a very close correlation between nodule development and the percentage composition of the plant, although from the standpoint of the need of carbohydrates for nitrogen fixation the better nodule development of the long-day plants might be regarded as being at least partly explained by their higher carbohydrate content. This same situation holds in the case of experiment III (variations of day-length in the fall). Because of the need of carbohydrates for nitrogen fixation, the higher carbohydrate content of the long-day plants might be regarded as explaining at least partly the better nodule development of these plants. But the other aspect of the relation of chemical composition to nodule development, discussed in connection with experiment I, that of the relationship of carbohydrates and nitrogen to root development, does not apply in experiment III any more than it did in experiment I. In each case the percentage of carbohydrates is less and the percentage of nitrogen is greater (this applies especially for the tops) in the short-day than in the long-day plants. This is the situation usually found in the case of highly vegetative, non-fruitful plants, which usually also have a smaller development of the roots in proportion to the tops. But, as has already been stated, both the short-day plants grown in the fall and the corresponding plants grown in the spring were less vegetative and had better development of roots in proportion to tops than the long-day plants. So neither the type of the plant nor nodule development, at least in so far as this is related to the carbohydrate-nitrogen relationship, seems to be explained by the percentage composition of the plant. Both seem to be related more closely to the amounts figured on an absolute basis. But there is need of greater fractionation of the carbohydrates and nitrogen. If

this had been done, there perhaps would have been found a closer correlation between the type of the plant and its percentage composition. However, since the roots of the short-day plants were, relative to the tops, better developed than those of the long-day plants, the carbohydrate-nitrogen relationship would still not explain the better nodule development of the long-day plants, unless we consider nodule development to be more in accord with the development of the tops than of the roots.

Others have noted similar differences in the type of soy bean plant when day-length was varied in the fall or winter (**11, 16**). ROSENFELS found that the long-day plants were highly vegetative and non-fruitful as compared with short-day plants, and were lower in carbohydrates in proportion to the nitrogen than the short-day plants. This is the situation usually found in this type of plant. NIGHTINGALE also found that long-day soy bean plants were vegetative and non-fruitful as compared with short-day plants. The short-day plants were higher in total nitrogen, nitrate nitrogen, total sugars, and starch and dextrin, but somewhat lower in hemicellulose than the long-day plants. NIGHTINGALE did not examine the nodules on the roots; ROSENFELS did, and found much better nodule development on the root of the short-day plants. This is directly opposite to my results. It would seem that further work needs to be done on the nodule development of long-day and short-day soy bean plants. ROSENFELS also found that the short-day plants were higher in carbohydrates, especially starch and dextrin, in proportion to the nitrogen, than the long-day plants. He considered that this was in accord with the better nodule development of these plants.

It is rather difficult to explain the phenomenon that long-day and short-day plants grown in the fall or winter were different in type from the corresponding plants grown in the spring or summer. One factor which should perhaps be considered is the difference in the length of day of the long-day plants in the two cases. The long-day plants in the spring were exposed to light about 13.5 hours per day, in the autumn about 16.3 hours. Although GARNER and ALLARD (**7**) have shown that certain varieties of soy beans are short-day plants, and continue vegetative if the day is kept too long, the difference in

day-length in the spring and autumn does not seem sufficient to account for the differences in the type of plant. The intensity of the light was of course less in the autumn than in the spring. But while, as shown by POPP (13), reducing light intensity causes twining of the soy bean, he, and also GARNER and ALLARD, found that reduction in light intensity had little if any effect on the time of flowering. There remains to be considered the quality of light. The quality of sunlight was different of course in the autumn from that in the spring; and since the long-day was secured by supplementing natural daylight with electric light, we have also to take into account the differences in the quality of electric light as compared with sunlight. Some work has been done showing that the quality of the light in which the plant is grown is very important. For example, POPP (12) grew soy beans and other plants in light of various qualities. Among the many effects noted when all rays shorter than 529 mμ were absent was a delay in the time of flowering, and also a reduction in the number of flowers produced. There is need of more work on the effects of light of various qualities on the development of plants. It seems likely that a difference in the quality of light was at least one important factor causing the plants grown in the autumn to be different in type from the plants grown in the spring. However, whatever was the external factor causing the markedly different type of plant when day-length was varied in the autumn, the carbohydrate-nitrogen relationship of the plants was not in accord with this difference in type.

Summary

1. The effects on growth and nodule development of the soy bean of varying certain factors that affect the amount of carbohydrate manufactured by the plants in the process of photosynthesis were studied. The factors varied were length of the exposure to light and the amount of photosynthetic tissue present, differences in the latter being secured by clipping the plants with different degrees of severity.

2. The amount of growth and nodule development was in direct proportion to the length of day and the degree of severity of the clipping.

3. Nodule development was correlated with percentage of carbo-

hydrates, especially acid-hydrolyzable material, of the plants. This correlation was much greater for the tops than for the roots. It is suggested that part of the decrease in nodule development resulting from decrease in photosynthesis is due to a lack of carbohydrates for the nitrogen fixation process.

4. No correlation was noted between the carbohydrate-nitrogen relationship of the plant and growth and nodule development in regard to the effect of this relationship on the development of the roots in proportion to the tops. The roots relative to the tops were better developed in the shorter than in the longer exposures. This lack of correlation may have been due to the fact that there was not sufficient fractionation of the carbohydrates and nitrogen.

5. Growth and nodule development of the plant were more closely correlated with the amounts of the chemical constituents when figured on an absolute basis than on a percentage basis. It is suggested that, with a decrease in photosynthesis there may be less growth of all parts of the plants, including the nodules, because of the smaller amount of food material present; but that the percentage composition of the resulting plants may be similar, or at least not different enough to account for the difference in the size of the plants. According to this viewpoint, the amounts of the chemical constituents figured on an absolute rather than a percentage basis may be more important in explaining the effects of the treatments.

6. The degree of greenness of the plants was in direct proportion to the length of exposure to light, and the phenomenon of etiolation was very prominent in the case of plants of the shorter exposures.

7. The plants resulting from a variation in day-length in the spring and from clipping were similar in type in regard to time of bud development, absence of twining, etc. The plants exposed to different day-lengths in the autumn were very different in type, the long-day plants being relatively vegetative, with a better development of tops in proportion to the roots, non-fruitful, and twining; the short-day plants being relatively non-vegetative, with a poorer development of tops in proportion to the roots, fruitful, and non-twining. However, nodule development of the autumn-grown plants was similar to that of the spring-grown, varying directly with the length of exposure to light.

8. Various external factors which might account for the fact that the plants exposed to different day-lengths were similar in the spring but different in the autumn are day-length, intensity of light, quality of light. Differences in the quality of the light may be an important factor.

9. Whatever were the external factors accounting for the differences in the types of plants when day-length was varied in the autumn, the carbohydrate-nitrogen relationship did not explain the differences. This may have been due to the fact that there was not sufficient fractionation of the carbohydrates and nitrogen.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BURK, D., The free energy of nitrogen fixation by living forms. Jour. Gen. Physiol. **10**:559–573. 1927.
2. BRYAN, O. C., Effect of different reactions on the growth and nodule formation of soy beans. Soil Sci. **13**:271–287. 1922.
3. CHRISTIANSEN-WENIGER, F., Der Energiebedarf der Stickstoffbindung durch die Knöllchenbakterien im Vergleich zu anderen Stickstoffbindungsmöglichkeiten und erste Versuche zur Ermittelung desselben. Centr. Bakt. Parasit. Abt. 2. **58**:41–66. 1923.
4. CLEMENTS, H. F., Hourly variations in carbohydrate content of leaves and petioles. BOT. GAZ. **89**:241–272. 1930.
5. ERNST, F. A., Fixation of atmospheric nitrogen. Van Nostrand Co. New York. 1928.
6. FRED, E. B., WHITING, A. L., and HASTINGS, E. G., Root nodule bacteria of Leguminosae. Wis. Agric. Exp. Sta. Res. Bull. 72. 1926.
7. GARNER, W. W., and ALLARD, H. A., Effect of relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agric. Res. **18**:553–606. 1920.
8. GIÖBEL, G., The relation of soil nitrogen to nodule development and fixation of nitrogen by certain legumes. N. J. Agric. Exp. Sta. Bull. 436. 1926.
9. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. Ore. Agric. Exp. Sta. Bull. 149. 1918.
10. LEONARD, L. T., A preliminary note on the relation of photosynthetic carbohydrate to nodule formation on soy beans. Jour. Amer. Soc. Agron. **18**:1012–1016. 1926.
11. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. Wis. Agric. Exp. Sta. Res. Bull. 74. 1927.

12. POPP, H. W., A physiological study of the effect of various ranges of wave length on the growth of plants. Amer. Jour. Bot. **13**:706–736. 1926.
13. ———, Effect of light intensity on growth of soy bean and its relation to the autocatalyst theory of growth. BOT. GAZ. **82**:306–319. 1926.
14. RANKER, E. R., Determination of total nitrogen in plants and plant solutions: a comparison of methods. Ann. Mo. Bot. Gard. **12**:367–380. 1925.
15. REID, MARY E., Growth of tomato cuttings in relation to stored carbohydrate and nitrogenous compounds. Amer. Jour. Bot. **13**:548–574. 1926.
16. ROSENFELS, R., The relation of length of day to growth expression and chemical constitution in the soy bean. Univ. Wis. Bachelor's Thesis, unpublished. 1927.
17. WHITING, A. L., A biochemical study of nitrogen in certain legumes. Ill. Agric. Exp. Sta. Bull. 179. 1915.
18. WILSON, J. K., Physiological studies of *Bacillus radicicola* of soy bean (*Soja max* Piper) and of factors influencing nodule production. Cornell Agric. Exp. Sta. Bull. 386. 1917.

FACTORS INFLUENCING FRUIT SETTING IN THE PECAN[1]

GUY W. ADRIANCE

(WITH SIX FIGURES)

Introduction

This study was undertaken to gain information on the effects of some cultural practices affecting fruit setting in the pecan. Since pollination is essential to complete fruit development in many flowering plants, it has been studied in association with the morphology and cytology of the developing nut.

MATERIALS AND METHODS

MORPHOLOGY AND CYTOLOGY.—For the study of the morphology and cytology of the developing pecan nut, collections of material for imbedding were made at frequent intervals during the early growing season. The first samples were taken from swelling buds, and the next from tips of growing shoots, before the pistillate flowers actually appeared. Following the appearance of the pistillate flowers, collections were made each day up to the time of receptivity and pollination; after which samples were taken at 4-hour intervals during the first day after pollination, then daily for 3 days, and then at 2-day intervals for 6 weeks. During the same period, samples which had been sacked and not pollinated were taken. In the later stages the shell of the nut was too hard for sectioning, so nuts collected after 4 weeks were trimmed with a knife to remove the hard shell. All material was killed and fixed in medium chromo-acetic solution, and sectioned according to the paraffin method The most satisfactory stains used were Delafield's haematoxylin, safranin-gentian violet, and acid fuchsin. The latter was satisfactory for staining pollen tubes.

[1] Submitted to the Graduate Faculty, Michigan State College, June 1929, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Published with the approval of the Director as Contribution no. 86 of the Technical Series of the Texas Agricultural Experiment Station (Sci. Paper no. 48, Div. of Hort.).

Some sections of very large fresh fruits were cut with a knife, killed in alcohol, cleared in acetone and cedar oil, and photographed with indirect light in thin glass cells filled with Canada balsam.

FRUIT DROPPING.—Field studies were undertaken with the idea of determining the amount of shedding of young pecan fruits, the time of shedding, and the factors influencing this condition. Preliminary observations and the work of other investigators indicated that the greater portion of the drop occurs early in the season. Records were taken of the dropping of the fruit during the period up to 6 weeks after time of pollination, on open-pollinated fruits and on fruits which were kept sacked until after all pollen was shed. Some further counts were made later in the season to check the relative importance of the early drop. The position of the dropped nuts on the peduncle was determined by the scar. These determinations were made twice a year, the proximal nut being counted as number "1" in all cases.

BLOSSOMING HABIT.—The first and last dates on which flowers were receptive was recorded for each tree. The period of receptivity was determined by the presence of a very perceptible viscous fluid on the stigmatic surface. The first and last dates on which pollen was shed were also recorded for each tree. The trees used for these records are in two orchards. The "old" orchard contains trees which were about 15 years old at the beginning of the study, and had been bearing moderately good crops. Seven varieties were used in this orchard, and from 3 to 12 trees of a variety, depending on the number available. In the "young" orchard the trees were 6 years old and beginning to bear well when the first data were taken in 1927. There were 10 varieties available in this orchard, and 8 or more trees of each variety.

METEOROLOGICAL DATA.—The data on temperature and precipitation were secured from the records of the Main Station Farm, Texas Experiment Station, which is about one-quarter of a mile from each orchard. Since, according to WOODROOF (**35**), SHUHART (**30**), and ISBELL (**10**), the staminate flowers are differentiated in the spring of the previous year and the pistillate flowers in the same spring in which they appear, it was not considered necessary to make records except for the late winter and spring, beginning January 1, of each

season, so as to give a sufficient margin before the beginning of growth.

The temperature records for the periods under consideration for each year have been converted into heat units, in order to have a definite basis for comparison. The remainder system of calculating the number of heat units above 40° F. was considered adequate for this study; the yearly total from January 1 to time of maturity of staminate and pistillate flowers was determined for each variety. The weekly, monthly, and seasonal distribution of heat units and precipitation for each spring season were also calculated so that the effect of these factors on dichogamy might be determined.

The work is concerned with the functioning of the plant over a period of years. The seasonal study is based upon a 5-year period, and an inspection of the meteorological records used shows that extremes of temperature and rainfall were encountered during this time.

Structure of flowers and fruits

The morphology of the species of *Carya* has been treated by BENSON and WELSFORD (3), NAWASCHIN (25), VAN TIEGHEM (33), ROWLEE and HASTINGS (28), DE CANDOLLE (7), MEEHAN (16), LUBBOCK (14), BRAUN (6), NICOLOFF (26), and others. BILLINGS (4) and WOODROOF (37) worked with the pecan specifically.

FLOWERING HABIT.—With regard to the production of staminate flowers, WOODROOF (35) and ISBELL (10) state that the catkins are formed within the bud in early spring, the process probably extending through April and May. In any case the staminate flowers are differentiated early in the growing season a full year previous to their appearance, and there is no chance for a deficiency of pollen due to retarded differentiation of stamens. In 1928 a Burkett tree at College Station had two clusters of pistillate flowers sacked after all catkins around the basal portion had been removed. When the sacks were later removed, it was noticed that one lateral bud on each of the new shoots had produced a group of catkins. Since these catkins were on current season's growth, they had certainly been differentiated just previous to their appearance. The catkins appeared normal but were too late to be of any value in producing pollen for the current season. The stigmas had all dried several weeks previously, while the catkins were still green and immature.

WOODROOF and WOODROOF (**36**) state that the pistillate flowers are normally differentiated from the terminal buds of the previous season but may be produced from lateral buds. SHUHART (**30**) gives three cases of false terminal buds which produced pistillate flowers, and maintains that the true terminal bud is strictly vegetative. It has been observed at College Station that few true terminal buds are produced, and the greater part of the pistillate flowers arise from lateral buds near the apical end of the shoot. Buds rendered subterminal in position by cutting back the dormant shoot have in practically all cases produced pistillate flowers.

Detailed study of the structure of the nut in various stages of development showed the following facts:

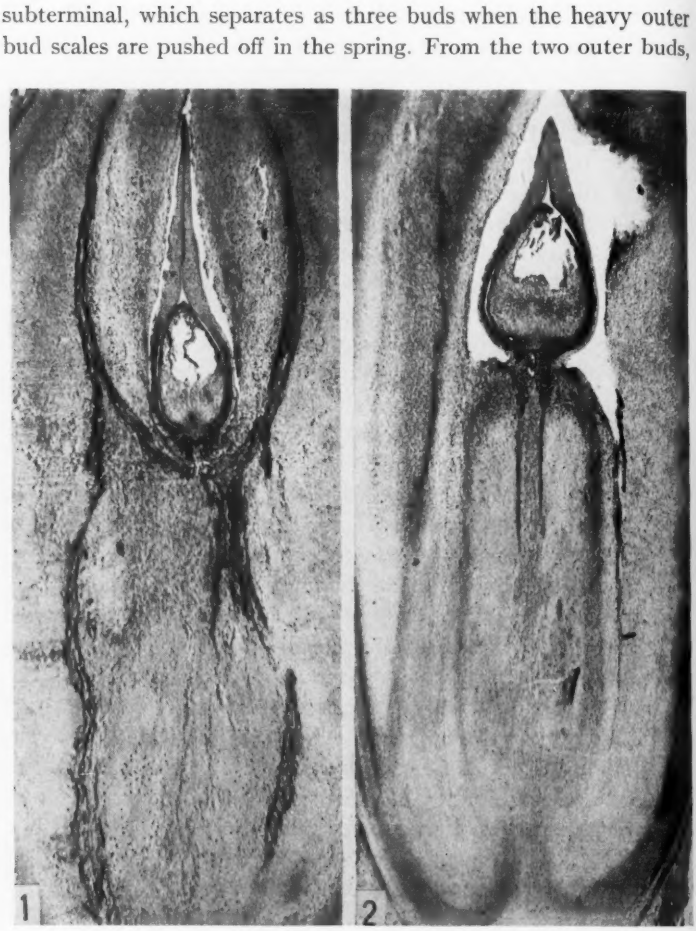
1. The middle septum of the nut is parallel to the axis of the inflorescence, and the plane of the stigmas is also parallel to this axis. In the walnut, although the plane is parallel to the axis, as in the pecan, the middle septum is perpendicular to this axis.

2. The peculiar branching of the vascular system of the walnut, as described by NICOLOFF (**26**) but not previously figured for the pecan, is clearly shown in this species (figs. 1, 2). NICOLOFF states that anterior and posterior to the ovule the placenta produces two bodies, in the form of horns, which are not an integument as has been represented. The ovule, exactly terminal, according to NICOLOFF, is supplied with symmetrical vascular connections from both sides by vessels (and also phloem) which come from the lateral bundles of the transverse partition, running to a point just below the ovule, and turning back to enter the integument. The development and the anatomy of the ovule, in his opinion, show that this organ has an axil dependence and not a carpellary dependence (cauline instead of foliar).

3. A vertical partition (middle septum) supports the orthotropous, sessile ovule, which has only one integument (figs. 1, 2). After fertilization, the cotyledons expand downward on either side of the partition, away from the micropyle. In the walnut (**26**) the ovary, at first unilocular, becomes at the time of fertilization quadrilocular in its basal portion, and also in its upper portion; this tendency is observed to a slight extent in the basal portion of the pecan.

4. The normal bearing habit of the pecan at College Station is the production from a single compound bud, lateral and usually

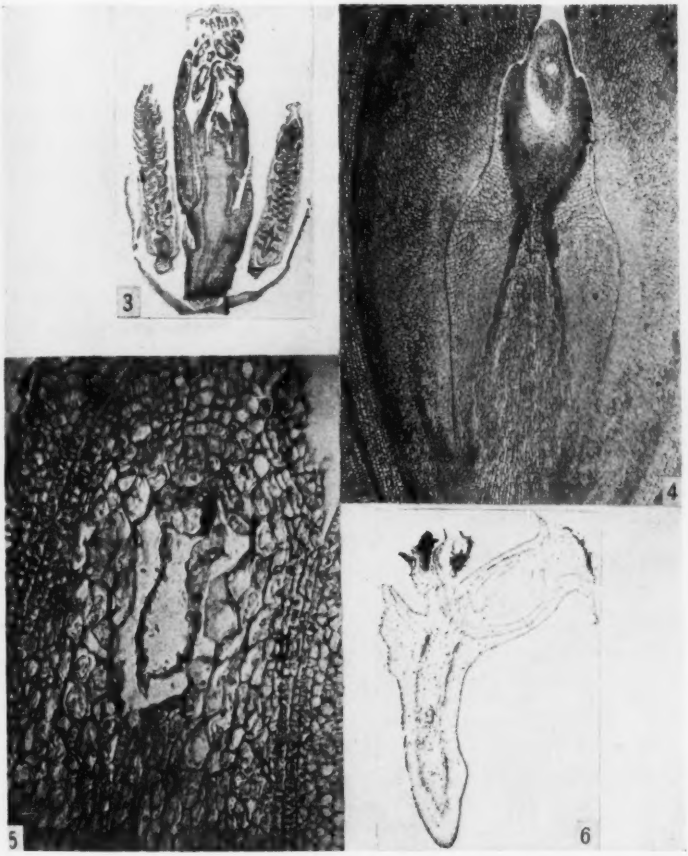
subterminal, which separates as three buds when the heavy outer bud scales are pushed off in the spring. From the two outer buds,



FIGS. 1, 2.—Fig. 1, longitudinal view of nut in plane of septum 6 weeks after pollination, showing gross morphology and portion of vascular connection of ovule; fig. 2, longitudinal view of nut perpendicular to septum, 6 weeks after pollination; ×18.

two clusters of three catkins each arise with a central shoot, coming from the bud between these two clusters, which terminates in a pistillate inflorescence. This is not in harmony with the common con-

ception that the staminate flowers arise from lateral buds and the pistillate flowers from terminal buds (fig. 3).



FIGS. 3–6.—Fig. 3, lateral bud of pecan at time of swelling in March, showing two clusters of catkins and central shoot with pistillate primordia, ×18; fig. 4, ovule at time of pollination, showing integument not yet inclosing nucellus, ×45; fig. 5, enlarged view of ovule 3 weeks after pollination, showing fertilized egg and disintegrated synergids, ×198; fig. 6, termination of pistillate flower cluster, showing one normal and two abortive flowers, which will shrivel and drop about time of pollination, ×18.

5. In the case of the Stuart pecan, small anther-like structures were observed on the flower axis at the bases of individual pistillate flowers. This condition was observed in two clusters, and no previous record of such structure has been found.

Pollination and fertilization

Chalazogamy, first observed by TREUB (**34**) in *Casuarina suberosa*, is recorded by BENSON (**2**) for *Betula*, *Alnus*, *Corylus*, and *Carpinus;* by NAWASCHIN (**24, 25**) for *Juglans regia* and *Corylus avellana;* by BILLINGS (**4**) in material of *Carya olivaeformis*, collected a few days after the withering of the anthers; and by WOODROOF and WOODROOF (**37**), also in the pecan. The writer's observations show that at the time of pollination the ovule is not completely surrounded by the integument, although it is later so inclosed. The pollen tube grows inward through the tissue of the stigma until near the central region of the style, and then grows downward, not in the stylar canal, but along the general course of its vascular tissues. It appears to go down to the chalazal region in various ways, usually in the wall of the ovary. In one case a pollen tube was unmistakably growing downward through the integument.

The ovule at the time of pollination is small and relatively undeveloped, and is not yet surrounded by the integument (fig. 4). Two weeks later the ovule is surrounded by the integument, the megaspore mother cell is discerned, and pollen tubes are present in the nucellus. There is considerable difference at this stage between the pollinated and unpollinated ovules. Although the evidence shows that considerable time elapses between pollination and fertilization, pollination is promptly followed by germination of the pollen grains, growth of the pollen tubes, and this in turn by progressive changes in the several ovarian and ovular tissues. Fig. 5 shows an ovule 3 weeks after pollination.

STERILITY

Sterility in plants has been the object of a considerable number of investigations, many of which indicate lack of pollination to be a factor of considerable importance. SAVASTANO (**29**) and STANCANELLI (**31**) record cases of sterility in pistache and filbert, both of

which are wind-pollinated nut fruits, where lack of pollination and fertilization was given as the cause.

There is evidence to show that sterility in the pecan is due to lack of viable pollen rather than to self- or inter-incompatibility. Regarding sterility in the pecan in Georgia, STUCKEY (**32**) states that self-sterility is due primarily to the interval in time between the receptive stage of the pistillate flowers and the shedding of pollen. This type of self-sterility, lack of self-pollination instead of true self-sterility or incompatibility, is not the usual condition in commercial fruit plants.

MORRIS (**21**) showed that eight varieties of the pecan gave an average of 74 per cent set when self-pollinated, and that most of the varieties gave a good set if the pollen was shed shortly before or during receptivity; but when shed early in the season it was not effective on the latest-maturing stigmas.

FACTORS AFFECTING FRUIT SHEDDING.—It is apparently possible to distinguish three distinct periods of dropping in the orchards at College Station: (1) at time of pollination; (2) 2–4 weeks after pollination; (3) at irregular intervals during the remainder of the growing period. With regard to the first condition, certain flower clusters shrivel and dry with the young nuts or flowers still attached to the peduncle. In other clusters, or in fact in nearly all clusters, there are one to three immature nuts at the apical end which shed along with the tip of the peduncle about the time of pollination. This last mentioned type of shedding, however, still leaves a sufficiently large cluster of nuts to produce a good crop. The shriveling of entire clusters has not been observed to any great extent, but the disturbed nutritive conditions following over-production might be responsible for such a condition, as explained by WOODROOF, WOODROOF, and BAILEY (**38**). The varieties Delmas and Burkett, used in this work, have borne good crops every year during the course of this study, and there has been no opportunity to observe "off" years.

The second type of dropping, referred to as the "May drop" (**38**), since it has been observed to occur 2–4 weeks following pollination in April and May, has proved of greatest significance in this study. WOODROOF and WOODROOF (**37**) state that fertilization occurs 2–4 weeks following pollination. The amount of this second

drop ranged from 11 to 52 per cent generally to 20 to 30 per cent, as indicated in table I.

The third drop later in the season, due presumably to environmental factors, does not amount to a large percentage of the total drop. The data in table I, showing the total drop to July 15 (about

TABLE I

AMOUNT OF DROP UNDER CONDITIONS OF OPEN POLLINATION 1927–1929

VARIETY	1927		1928		1929	
	Total nuts	Percentage dropped	Total nuts	Percentage dropped	Total nuts	Percentage dropped
	Four weeks after pollination					
Schley	119	11	.....	.....	.....	.....
Texas Prolific	301	10	303	22	.....	.....
Delmas	363	23	527	27	2100	17
Burkett	383	19	498	26	1980	41
Rome	209	52	.....	.....	.....	.....
Success	87	17	.....	.....	.....	.....
San Saba	.....	.....	388	23	.....	.....
Stuart	.....	.....	328	23	.....	.....
Moneymaker	.....	.....	420	20	.....	.....
All varieties	.....	22	.....	23	.....	29
	To July 15					
Delmas	.....	.....	527	35	2100	24
Burkett	.....	.....	498	36	1980	48
San Saba	.....	.....	388	26	.....	.....
Stuart	.....	.....	328	28	.....	.....
Texas Prolific	.....	.....	303	26	.....	.....
Moneymaker	.....	.....	420	23	.....	.....
All varieties	.....	.....	.....	29	.....	36

10 weeks after pollination), and the work of BILSING (5) which shows the drop for the entire summer, indicate that the early or "May" fall may account for the greater part of the seasonal drop.

In the case of the unpollinated nuts, which were sacked before any pollen was shed and kept so until no more pollen was available, there appeared to be about the same amount and character of drop during the first 3 weeks following pollination. After this time, however, the rate of dropping increased rapidly and the condition at the

end of the fourth week is shown in table II. There were no unpollinated Burkett nuts to be found for collection the fifth week after pollination time, and, although the Delmas nuts persisted somewhat longer, there were only twelve of these unpollinated nuts after the sixth week. The latter nuts persisting at this time showed clearly defined abscission layers in all cases examined.

ABSCISSION.—Two general types of shedding were observed in Delmas and Burkett. In one case, which is less frequently observed,

TABLE II

EFFECT OF POLLINATION AND LACK OF POLLINATION ON EARLY DROP OF PECANS, 1928

VARIETY	TOTAL NO. OF CLUSTERS	PERCENTAGE OF CLUSTERS IN WHICH NUMBER OF NUTS REMAINING AFTER 4 WEEKS WAS						
		0	1	2	3	4	5	6
		Sacked and not pollinated						
Delmas	392	32	7	15	20	18	7	1
Burkett	373	69	5	6	9	7	3	1
		Pollinated						
Delmas	253	0	7	21	31	28	10	3
Burkett	250	6	5	11	28	34	13	3

the entire peduncle dries up, although the nuts persist. This occurs in the early stages of development of the cluster, usually within a few days after pollination. The nuts never attain the size they do in the case mentioned later. Shedding of the entire peduncle is due in nearly all cases to mechanical injury, such as insect damage or breakage by wind.

The more common occurrence is the shedding of the individual flowers from the peduncle. The nuts in the cluster do not shed all at the same time, although there does not appear to be any regular order for the drop. Even after all the nuts have dropped from the peduncle, it persists in a green condition for a period of several weeks. The evidence presented by the basal region of unpollinated nuts indicates that the abscission layer is well defined within 3 weeks after receptivity. The abscission appears to be produced in the char-

acteristic manner, as described by HANNIG (**9**), LLOYD (**12, 13**), and NAMIKAWA (**22, 23**) for various nut fruits, not including the pecan. The walls of the cells in the separation layer are softened and the cells grow longitudinally, producing considerable displacement and disruption of the tissues.

The data presented in tables I and II indicate that the latter type of drop, the shedding of the separate nuts from the peduncle, follows pollination. The few unpollinated nuts in the controlled experiments which persisted after the 6-week period may actually have been pollinated accidentally, or may have developed parthenocarpically. There are sometimes a few seedless pecans, which might be accounted for on this basis.

EFFECT UPON SHEDDING OF PEDUNCLE POSITION.—Preliminary studies were made to determine the relative ability of the nuts in different positions on the peduncle to set and mature. A large number of clusters were treated in the following ways: (1) pollination of two basal flowers only; (2) pollination of two terminal flowers only; (3) pollination of two terminal flowers and snipping off basal flowers; (4) pollination of one basal and one terminal flower. Considerable difficulty was encountered in this work, owing to the lightness of the pollen, which made it almost impossible to keep it from the other flowers in the cluster. A number of pollinations were made as outlined, after the nuts to be left unpollinated had been covered with small gelatin capsules. Because of high winds, most of these capsules were shaken off and the nuts pollinated from loose pollen in the bags. It was also attempted to kill the stigmatic surface of the nuts not to be pollinated with osmic acid. The results in both cases were of questionable value and are not included.

Later studies on open pollinated clusters, however, show that there is a rather definite relationship between the position of the nut in the cluster and the probability of its shedding. Table III indicates that in clusters of varying numbers the basal nut or flower is much more likely to be dropped than any other. The position next to the basal is the one showing the next largest percentage of drop, and the apical nut or flower third. The percentages of drop in the respective positions are 13, 7, and 4.5. Those nuts in intermediate positions are much less likely to drop. These data are not in accord with

those given by WOODROOF, WOODROOF, and BAILEY (38), who found the number of nuts to the cluster influenced the position of shedding. It is frequently observed with the pecans at College Station, how-

TABLE III

RELATION BETWEEN POSITION OF NUT IN CLUSTER AND SHEDDING

VARIETY	NO. OF NUTS	PERCENTAGE SHED TO JULY 2, 1928					
		1 (basal)	2	3	4	5	6
		Clusters of 6 nuts					
Delmas	264	12	8	3	2	3	6
Burkett	156	14	9	2	4	6	4
San Saba	12	18	8	0	0	0	0
Moneymaker	48	14	4	0	2	0	2
Total	480	13	8	2	3	4	5
		Clusters of 5 nuts					
Delmas	215	13	9	4	1	5	
Burkett	250	14	10	5	4	5	
San Saba	90	12	2	3	1	2	
Stuart	50	16	6	6	2	10	
Moneymaker	110	10	3	0	0	4	
Total	715	13	7	4	2	5	
		Clusters of 4 nuts					
Delmas	40	17	5	2	7		
Burkett	76	20	8	4	4		
San Saba	204	11	8	1	5		
Stuart	144	8	5	5	5		
Texas Prolific	116	20	4	1	3		
Moneymaker	200	13	7	3	3		
Total	780	13	6	3	4		
		Clusters of 3 nuts					
Delmas	9	11	0	11			
Burkett	15	20	7	0			
San Saba	84	12	13	5			
Stuart	87	12	6	6			
Texas Prolific	123	16	5	4			
Moneymaker	54	7	11	2			
Total	372	13	8	4			

ever, that the basal nut does not always complete its development in time for pollination, and the heavy shedding from this position may be attributed to lack of pollination.

Within the pistillate inflorescence, the different flowers of the cluster are at the same stage of development at the time of pollination, except for the smaller ones noted later. It has not been possible to fix a different time of receptivity for the different flowers of normal size in the cluster. According to WOODROOF and WOODROOF (**37**), the basal flowers are differentiated first, and there are always some undeveloped flowers at the apex of each cluster. These flowers are normal but not far enough advanced to be receptive, and are shed soon after the period of pollination (fig. 6). It has been observed in several cases, especially with Texas Prolific, that there are sometimes one or two immature flowers at the base of the cluster; these have always been observed to fall without reaching the stage for pollination. WOODROOF and WOODROOF (**37**) illustrate this characteristic, but state that such flowers may develop into nuts.

A careful consideration of the preceding data seems to warrant the following interpretations: (1) The so-called "May" drop of pecans occurring 2–4 weeks after pollination accounts for the greater portion of the total drop, except those which are damaged by insects and diseases, or drop later as the result of drought. (2) This "May" drop appears to be the result of lack of fertilization of the flowers, due primarily to lack of pollination, since it coincides with the drop of sacked unpollinated flowers. (3) A proper supply of pollen at the time the pistillate flowers are receptive is the primary requisite for setting and development of the nuts.

In view of the fact that pollination seems to be the limiting factor in determining the set of fruit in the pecan, a consideration of the factors influencing pollination is of primary importance. Since dichogamy nearly always occurs in monoecious or dioecious plants, investigations of this condition were made in the case of the pecan.

DICHOGAMY

The factors of primary importance in the consideration of dichogamy are the character and extent of the difference in time of maturity of pistillate and staminate flowers, and the factors which

influence this difference in maturity. KERNER and OLIVER (**11**) state that all monoecious plants are protogynous, although other workers (**15–20, 27**) have shown that protogyny is not always the rule in the nut fruits. STUCKEY (**32**) divided the pecan varieties into two groups on the basis of dichogamy, stating that in group I the pistillate flowers of most varieties become receptive at the same time that the

TABLE IV

RANGE OF MATURITY OF PECAN FLOWERS, 1925–29 (DAYS AFTER MARCH 31; E.G., APRIL 10=10, MAY 10=40)

VARIETY	STAMINATE					PISTILLATE				
	1925	1926	1927	1928	1929	1925	1926	1927	1928	1929
	Old orchard									
Texas Prolific	9–16	21–26	10–15	20–30	9–14	11–22	31–40	17–21	24–34	9–15
San Saba	9–15	27–32	10–16	24–34	11–20	11–23	33–38	17–24	28–39	11–21
Delmas	14–21	31–38	17–23	31–40	20–25	8–15	32–37	19–20	23–32	10–18
Stuart	14–24	30–38	18–30	35–43	21–26	12–24	34–40	19–22	28–36	10–20
Moneymaker	17–21	31–37	14–20	29–37	16–21	10–17	31–35	14–15	24–30	11–19
Bolton	9–17	28–34	13–20	27–35	16–22	7–12	26–28	13–14	23–28	9–15
Rome	9–13	24–28	10–15	24–32	11–19	8–13	30–35	18–20	25–35	9–15
	Young orchard									
Schley			17–28	33–39	19–24			19–21	24–34	10–18
Moneymaker			14–21	28–36	20–27			12–13	27–42	10–18
Success			26–28	32–39	17–22			26–30	25–34	13–19
Alley			11–21	24–32	11–16			22–25	31–45	14–20
Moore			8–14	22–30	7–13			21–26	25–44	10–14
Burkett			17–28	32–40	20–26			18–21	24–32	9–17
Onliwon			11–19	26–33	14–18			24–30	28–44	11–16
Atwater				33–40	20–25				25–32	10–14
Western Schley			15–20	29–34	14–19			24–28	32–46	11–16
Delmas			17–30	32–41	20–26			19–21	25–37	10–20

staminate flowers shed their pollen, while in group II the pistillate flowers become receptive 2–10 days before the staminate flowers shed their pollen.

The blossoming data in table IV show that the type of dichogamy in the pecan is not always fixed, and data in table V make it evident that there is a strong tendency in certain seasons toward protandry and in others toward protogyny. Some of the most important commercial varieties, as Delmas, Schley, Stuart, and Burkett, respond

to these seasonal tendencies. It is also shown (table V) that there is a group of varieties which have a positive tendency toward protandry, which tendency has not been observed by previous investigators. Texas Prolific, San Saba, Moore, and Alley were protandrous every year. Another group shows a positive trend toward protogyny, although this is not so strongly marked as in the protandrous

TABLE V

CHARACTER AND EXTENT IN DAYS OF DICHOGAMY; + INDICATING PROTOGYNY AND − PROTANDRY

VARIETY	1925	1926	1927	1928	1929
	Old orchard				
Texas Prolific	2−	10−	7−	4−	0
San Saba	2−	6−	9−	4−	0
Delmas	6+	1−	2−	6+	10+
Stuart	2+	4−	1−	6+	11+
Moneymaker	7+	0	0	5+	5+
Bolton	2+	2+	1+	4+	7+
Rome	1+	6−	8−	2+	4−
	Young orchard				
Schley	.....	.....	2−	9+	9+
Moneymaker	.....	.....	2+	1+	10+
Success	.....	.....	0	7+	4+
Alley	.....	.....	11−	7−	3−
Moore	.....	.....	13−	3−	3−
Burkett	.....	.....	1−	8+	11+
Onliwon	.....	.....	13−	2−	3+
Atwater	.....	.....	.....	8+	10+
Western Schley	.....	.....	7−	3−	3+
Delmas	.....	.....	2−	7+	10+

group. Moneymaker, Success, and Bolton were protogynous in almost every case through the entire period; Moneymaker in 1926 and 1927, and Success in 1927, matured staminate and pistillate flowers on the same date.

In view of these facts, it seems advisable to depart from previous classifications, and make three groups of pecan varieties as regards dichogamy: protandrous, fluctuating, and protogynous.

SEASONAL TENDENCIES.—The behavior of the fluctuating varieties, as well as the tendency of the positive varieties toward over-

lapping in blossoming, indicate that the seasons of 1925, 1928, and 1929 exerted some influence toward protogyny; the seasons of 1926 and 1927 toward protandry. It was considered advisable to ascertain whether these tendencies might be associated with definite conditions of environment, as suggested by MEEHAN (**16, 18**).

The critical factors which might cause a difference in maturity of the flowers were considered to be temperature and rainfall, although

TABLE VI

WEEKLY, MONTHLY, AND SEASONAL ACCUMULATION OF HEAT UNITS ABOVE 40° F. (MEAN) AT COLLEGE STATION, 1925–29

DATE		1925	1926	1927	1928*	1929
January	7	72.5	51.0	113.0	20.0	40.5
	14	123.0	74.0	166.0	136.5	110.0
	21	159.0	188.5	269.0	279.5	229.0
	28	231.0	207.0	326.0	228.0	321.0
February	4	320.5	309.5	490.5	460.0	368.0
	11	483.5	432.5	602.5	570.0	387.5
	18	597.5	592.5	729.0	625.5	445.5
	25	781.5	728.5	857.5	686.5	494.0
March	4	901.5	853.0	923.0	781.5	599.5
	11	1103.0	982.0	1069.0	961.5	750.0
	18	1272.5	1067.0	1224.0	1119.0	873.0
	25	1441.0	1249.0	1322.5	1241.5	1076.5
April	1	1644.0	1316.5	1524.0	1422.0	1314.0
	8	1843.5	1487.5	1742.0	1590.0	1573.0
	15	2081.0	1621.5	1977.5	1684.0	1786.0
	22	2358.0	1799.5	2173.0	1857.5	2016.0
	29	2618.5	1082.0	2340.0	2020.5	2261.5
	30	2632.5	2010.0	2372.0	2079.5	2302.0

* Leap year, February 29 included in figures and date advanced one day, beginning March 3.

wind and atmospheric humidity probably have considerable influence upon duration of receptivity of the stigmas and shedding of the pollen.

In the consideration of the effect of temperature, the number of heat units above 40° F. mean was recorded for the first 4 months of each year, as described in the first part of this work. These figures were arranged to show weekly, monthly, and seasonal accumulations during the entire period (table VI). As shown by the totals on April 30, the year 1925 had the greatest number of heat units; 1927 and

1929 were about equal and considerably fewer than 1925; and 1926 and 1928 had still fewer than the two last mentioned.

The monthly rainfall for the entire period and the weekly accumulations are shown in table VII. The rainfall in the three protogynous seasons was considerably less than in the protandrous seasons. For the protogynous seasons, 1925 had 4 inches, 1928 had 9 inches,

TABLE VII

WEEKLY, MONTHLY, AND SEASONAL RAINFALL AT COLLEGE STATION (INCHES) 1925–29

DATE	1925	1926	1927	1928	1929
January					
7	0.00	1.55	0.00	0.29	2.53
14	1.12	1.58	1.03	0.29	4.50
21	1.69	3.97	1.30	0.35	4.84
28	1.69	4.32	1.63	0.39	5.09
February					
4	1.72	4.37	1.63	1.47	5.24
11	1.77	4.37	3.44	1.61	5.90
18	1.77	4.58	3.58	2.35	5.95
25	2.17	4.58	3.58	4.70	6.26
March					
4	2.34	5.12	7.88	4.70	1.92
11	2.34	7.80	9.07	5.97	7.04
18	2.67	8.48	9.07	6.61	7.99
25	2.67	10.46	10.76	6.69	8.92
April					
1	3.55	12.62	10.76	6.99	8.94
8	3.55	12.69	10.77	7.64	9.71
15	3.55	13.96	14.69	9.74	10.68
22	3.55	16.37	16.91	9.83	10.69
29	4.17	16.61	17.39	9.83	10.69

and 1929 had 10 inches. For the protandrous years, 1926 had 17 inches, and 1927 had 17 inches. The weekly accumulations give a better idea as to the distribution of this rainfall.

When the combined effect of temperature and rainfall is considered, it may be observed that these two factors compensate for each other to some extent. The season of 1925, which was very hot and very dry, was not so strongly protogynous as the season of 1928, which was much cooler and had more rainfall. In the same way the season of 1929, which was even more strongly protogynous than 1928, had very little more rainfall than 1928, and was much cooler up to the early part of April. The greater total of heat units is ac-

counted for by the sustained high temperature in April, after many of the trees had blossomed.

In the two protandrous seasons of 1926 and 1927, however, there was no observed difference in blossoming which might be attributed to the difference in heat unis. The heavy rainfall is the only outstanding factor which is common to these two seasons.

When the heat units to date of maturity are considered, as shown in table VIII, two facts stand out:

1. On the basis of the means, there is a much greater variation in any one year between varieties in number of heat units to maturity of staminate flowers than to maturity of pistillate flowers. To express the same behavior in a different way, there is less difference in the date of blossoming of pistillate flowers than of staminate. This same difference is apparent in both the old and the young orchards, where both age and variety of the trees are different. The significance of these differences is greater from the fact that Delmas and Moneymaker, the only two varieties occurring in both orchards, check closely in their requirements.

2. The pistillate flowers vary much more from year to year in their total requirements than do the staminate flowers, the greatest coefficient of variability for staminate flowers, 6.1, being less than the least coefficient for pistillate flowers, 6.7. The actual range in the coefficient of variability for the staminate flowers of the different varieties is from 2.3 to 6.1, and for the pistillate flowers, 6.7 to 9.5. As shown in table IX, the odds in favor of the significance of this difference are 999 to 1.

With regard to all the observations recorded, it may be stated that the conditions of environment in the spring exert considerable influence on blossoming of the pecan. In general it appears that, although the date of maturity of both staminate and pistillate flowers is influenced by favorable conditions for growth in the spring, the staminate flowers respond more readily than the pistillate flowers. This fact would indicate a possibility that seasons favorable for early growth might be favorable for protandry. ADRIANCE (1) has previously presented data showing some effects of spring temperatures in this respect.

In confirmation of this idea, it may be stated that since the pistillate flowers of the pecan are differentiated in the same spring that

TABLE VIII

HEAT UNITS TO DATE OF MATURITY OF FLOWERS

VARIETY	STAMINATE								PISTILLATE							
	1925	1926	1927	1928	1929	Mean	S.D.	C.V.	1925	1926	1927	1928	1929	Mean	S.D.	C.V.
	Old orchard															
Delmas	2010	2010	2004	2079	1949	2010 ±14	46.1	2.3	1811	2040	2068	1926	1638	1896 ±53	176.7	9.2
Moneymaker	2117	2010	1924	2020	1816	1977 ±34	113.2	5.7	1875	2010	1924	1905	1667	1876 ±38	127.3	6.7
Texas Prolific	1843	1746	1786	1789	1604	1753 ±27	90.4	5.1	1908	2010	2004	1905	1604	1885 ±50	165.5	8.8
San Saba	1843	1904	1786	1905	1667	1821 ±29	99.2	5.4	1908	2070	2068	2002	1667	1943 ±50	167.8	8.6
Stuart	2010	1982	2035	2199	1986	2042 ±27	89.3	4.3	1939	2100	2068	2020	1638	1953 ±56	186.2	9.5
Bolton	1843	1930	1889	1980	1816	1891 ±20	65.9	3.4	1782	1881	1856	1885	1604	1801 ±35	117.9	6.5
Rome	1843	1820	1786	1980	1667	1821 ±33	112.7	6.1	1811	1982	2035	1926	1604	1871 ±51	171.1	9.1
	Young orchard															
Delmas	.....	.....	2004	2119	1949	2024			.....	.....	2068	1926	1638	1877		
Moneymaker	.....	.....	1924	2002	1949	1958			.....	.....	1856	1980	1638	1825		
Schley	.....	.....	2004	2139	1909	2017			.....	.....	2068	1905	1638	1870		
Success	.....	.....	2219	2119	1845	2061			.....	.....	2219	1926	1725	1957		
Alley	.....	.....	1822	1905	1667	1798			.....	.....	2162	2079	1756	1999		
Moore	.....	.....	1708	1857	1540	1702			.....	.....	2138	1926	1638	1901		
Burkett	.....	.....	2004	2119	1949	2024			.....	.....	2035	1905	1638	1859		
Onliwon	.....	.....	1822	1951	1756	1843			.....	.....	2185	2002	1667	1951		
Atwater	.....	.....	.....	2139	1949	2044			.....	.....	.....	1926	1638	1782		
Western Schley	.....	.....	1952	2020	1756	1909			.....	.....	2185	2109	1667	1987		

they appear, but staminate flowers are differentiated the previous spring, under these conditions apparently a "quick" season might mature the staminate flowers earlier than the pistillate. A cold or dry season, on the other hand, might retard the opening of the staminate flowers long enough for the pistillate to differentiate.

TABLE IX

SIGNIFICANCE OF COEFFICIENTS OF VARIABILITY IN TABLE VIII

C.V. STAMINATE A	C.V. PISTILLATE B	B−A	D	D^2
5.1	8.8	3.7	0.0	0.0
5.4	8.6	3.2	−0.5	0.25
2.3	9.2	6.9	3.2	10.24
4.3	9.5	5.2	1.5	2.25
5.7	6.7	1.0	−2.7	7.29
3.4	6.5	3.1	−0.6	0.36
6.1	9.1	3.0	−0.7	0.49
		7)26.1	+4.7	7)20.88
		3.7	−4.5	2.98

$$P = \sqrt{2.98}$$

$$= 1.726$$

$$Z = \frac{3.7}{1.726}$$

$$= 2.1436$$

odds 999 to 1.

A further consideration is the fact that pistillate flowers are borne on the terminal part of new shoots, and a season favorable to strong vegetation might delay their development.

Summary

1. The pistillate flower of the pecan consists of an orthotropous ovule, surrounded by a single integument. The portion which becomes the shell consists of two carpels, which are transverse on the axis of the inflorescence. The 4-valved husk is developed from the lower portions of the calyx lobes. The flowers are sessile on the peduncle, and are borne in clusters usually of two to six.

2. The pollen tube grows down through the style and ovary wall or integument to the base of the ovule, and returns through the chalaza and nucellus to the embryo sac, fertilization occurring about 4 weeks after pollination.

3. There is a definite drop of young nuts about 4 weeks after the

time of pollination, and this drop accounts for over 75 per cent of the seasonal drop. It appears to be due to lack of pollination.

4. The varieties tested show no evidence of self-incompatibility or inter-incompatibility. Good sets of fruit were obtained from any variety of pollen available when the flowers were receptive.

5. The period of maturity of staminate and pistillate flowers of the pecan do not often coincide. This condition of dichogamy may be complete or incomplete, and the special type may be protandry or protogyny. Some varieties, such as Moore, Alley, Texas Prolific, and San Saba, have been protandrous every season, and have had pollen available in time to pollinate the earliest flowers of any variety. Some varieties, especially Moneymaker, Bolton, and Success, have been protogynous or overlapping slightly every year, and have been dependent upon other varieties for pollination in almost every case. A group of varieties, including Delmas, Burkett, Schley, and Stuart, which are frequently recommended for planting together, have been protandrous or overlapping only two years in five. These leading varieties in Texas need other varieties near them to insure availability of early pollen.

6. Certain seasons have been favorable to protandry and others to protogyny. The data on temperature and rainfall for the spring seasons indicate that moisture and high temperature in this period favor early maturity of the staminate flowers, and cool, dry seasons favor earlier maturity of the pistillate flowers. The warm seasons of heavier rainfall advance maturity of both the staminate and pistillate flowers, but the staminate flowers of any one variety are less variable in their requirement of heat units.

7. It may be said that the early dropping of fruits in the pecan is due primarily to lack of pollination, and this, in turn, is due to dichogamy. Certain varieties (Moore, Alley, Texas Prolific, and San Saba) have proved to be reliable in the production of pollen at an early date, and some of these varieties should be used in every pecan orchard.

The writer desires to express appreciation to Professors V. R. GARDNER, F. C. BRADFORD, and J. W. CRIST, of the Department of Horticulture, Michigan State College, who directed the research and gave much valuable criticism. Acknowledgment is due to Dr. H. P.

TRAUB, Division of Horticulture and to Dr. P. C. MANGELSDORF, Division of Agronomy, Texas Agricultural Experiment Station, for stimulating and constructive criticism on the completed manuscript.

A. AND M. COLLEGE OF TEXAS
COLLEGE STATION, TEXAS

[*Accepted for publication April 10, 1930*]

LITERATURE CITED

1. ADRIANCE, GUY, A preliminary report on dichogamy in the pecan. Proc. Amer. Soc. Hort. Sci. 1927: 95–97. 1927.
2. BENSON, M., Contributions to the embryology of the Amentiferae. Part I. Trans. Linn. Soc. Bot. Ser. vols. 2. **111**:409–424. 1894.
3. BENSON, M., and WELSFORD, E. J., The morphology of the ovule and female flower of *Juglans regia* and of a few allied genera. Ann. Botany **23**:625–633. 1909.
4. BILLINGS, F. H., Chalazogamy in *Carya olivaeformis*. BOT. GAZ. **35**:134–135. 1903.
5. BILSING, S. W., The life history and control of the pecan nut case bearer. Texas Agric. Expt. Sta. Bull. 328. 1926.
6. BRAUN, A., Über den inneren Bau der Frücht der Juglandeen. Bot. Zeit. **30**: 371–375. 1872.
7. DE CANDOLLE, C., Mémoire sur la famille des juglandées. Ann. Sci. Nat. Bot. Ser. IV. **18**:1862.
8. CONRAD, A. H., A contribution to the life history of *Quercus*. BOT. GAZ. **29**:408–418. 1900.
9. HANNIG, E., Untersuchungen über das Abstossen von Blüten unter dem Einfluss äusserer Bedingungen. Ztschr. Bot. **5**:417–469. 1913.
10. ISBELL, C. L., Growth studies of the pecan. Ala. Agric. Expt. Sta. Bull. 226. 1928.
11. KERNER, A., and OLIVER, F. W., Natural history of plants. **2**(1):312–313. New York. 1896.
12. LLOYD, F. E., Abscission in *Mirabilis jalapa*. BOT. GAZ. **61**:213–230. 1916.
13. ———, Abscission of fruit in *Juglans californica quercina*. Trans. Roy. Soc. Canada 14. Sec. V. 17–22. 1920.
14. LUBBOCK, SIR JOHN, On the fruit and seed of the Juglandaceae. Jour. Linn. Soc. Bot. **28**:247–254. 1891.
15. MEEHAN, THOMAS, Dimorpho-dichogamy in *Juglans* and *Carya*. BOT. GAZ. **5**:11. 1880.
16. ———, Influence of temperature on the separate sexes of flowers (abs.). Acad. Nat. Sci. Phil. Proc. 1885. pp. 117. 1886.
17. ———, Dichogamy and its significance. Acad. Nat. Sci. Phila. Proc. 1888. pp. 391. 1889.
18. ———, On the varying character of dichogamy in flowers of *Corylus avellana*. Acad. Nat. Sci. Phila. Proc. 1890. 268–269. 1891.

19. ———, Sex in flowers: *Corylus rostrata*. Acad. Nat. Sci. Phila. Proc. 1899. 84–86. 1900.
20. MOLISCH, H., Forcing plants by warm baths. Umschau 12, 771–773. 1908. Esr. XX. 641. Abs. in Sci. Amer. Sup. 66 1908. no. 1715. pp. 298. Abs. in Expt. Sta. Rec. **20**:640–641. 1909.
21. MORRIS, H. F., A study of self-sterility in the pecan. Unpublished thesis, Texas A. and M. College. 1925.
22. NAMIKAWA, J., Über die vorzeitige Abstossung der jungen Früchte von Malus communis. Jour. Coll. Agric. Hokkaido Imp. Univ. **11**:1–21. 1922.
23. ———, Contributions to the knowledge of abscission and exfoliation of floral organs. Jour. Coll. Agric. Hokkaido Imp. Univ. **17**:63–131. 1926.
24. NAWASCHIN, S., Neue Ergebnisse über die Embryologie der Haselnuss (*Corylus avellana*). Bot. Centralb. **63**:104–106. 1895.
25. ———, Ein neues beispiel der chalazogamie. Bot. Centralb. **63**:353–357. 1895.
26. NICOLOFF, M. TH., Sur le type floral et le développement du fruit des Juglandées. Jour. Bot. [Paris] **18**:134–152; 380–385. 1904; **19**:63–68; 69–84. 1905.
27. PRINGLE, C. G., Dimorpho-dichogamy in *Juglans cinerea* L. BOT. GAZ. **4**:237. 1879.
28. ROWLEE, W. W., and HASTINGS, G. T., The seeds and seedlings of some Amentiferae. BOT. GAZ. **26**:349–353. 1898.
29. SAVASTANO, GUILIO, Sulla improduttività del pistacchio in Sicilia. Ann. R. Staz. Sper. Agrum. e Frutt. Acireale **8**:57–64. 1926.
30. SHUHART, D. V., The morphological differentiation of the pistillate flowers of the pecan. Jour. Agric. Res. **35**:687–696. 1927.
31. STANCANELLI, M., R. La coltivazione del nocciuolo nella provincia di Messana. Ann. R. Staz. Sper. Agrum. e Frutt. Acireale **2**:139–140. 1914.
32. STUCKEY, H. P., The two groups of varieties of the Hicoria pecan and their relation to self-sterility. Ga. Agric. Expt. Sta. Bull. 124. 127–146. 1916.
33. VAN TIEGHEM, P., Anatomie de la fleur femelle et du fruit du noyer. Soc. Bot. France Bull. **16**:412–419. 1869.
34. TREUB, M., Sur les Casuarinées et leur place dans le système naturel. Ann. Jard. Bot. Buitenzorg **10**:177–187. 1891.
35. WOODROOF, J. G., The development of pecan buds and the quantitative production of pollen. Ga. Agric. Expt. Sta. Bull. 144. 134–161. 1924.
36. WOODROOF, J. G., and WOODROOF, N. C., Fruit bud differentiation and subsequent development of the flowers in the Hicoria pecan. Jour. Agric. Res. **33**:7. 677–685. 1926.
37. ———, The development of the pecan nut (Hicoria pecan) from flower to maturity. Jour. Agric. Res. **34**:1049–1063. 1927.
38. WOODROOF, H. G., WOODROOF, N. C., and BAILEY, J. E., Unfruitfulness of the pecan. Ga. Agric. Exp. Sta. Bull. 148. 1928.

RESPIRATION OF THE SHOOT AS AFFECTED BY TEMPERATURE CHANGES OF THE ROOT[1]

W. H. MICHAELS

(WITH FIVE FIGURES)

Introduction

The effect of temperature upon plant respiration has been extensively studied since the work of DE SAUSSURE and GAREAU, both of whom simply demonstrated that the rate of respiration rose with an increase in temperature. CZAPEK (**8**), KUIJPER (**13**), and FERNANDES (**10**) give extensive reviews of the literature dealing with this phase of respiration. In general it may be said that the rule of Van't Hoff holds for plant respiration between certain limits of temperature, usually considered to be about 5° –25° C. There are a number of exceptions to this rule, however, for example, BENNETT and BARTHOLOMEW (**2**) found the respiration of potato tubers to be greater at 0° than at 5° C., also that the rule appeared to hold in this case for the rather wide limits of 5°–45° C.

The rate of respiration is by no means constant, even at temperatures which may be optimum for the growth of the organ under investigation. MATTHAEI (**18**), KUIJPER (**13**), and FERNANDES (**10**) have reported marked fluctuations in the respiratory rate, especially at temperatures above 30° C. FERNANDES pointed out that the temperature at which these fluctuations become apparent varies with the age of the seedling, and that the response to temperature varies with different lots of seedlings, even though they be of the same age. BLACKMAN and PARIJA (**4**), working with individual apples at 22° C., were forced to use two "contour lines" in the graphs presenting their data, in order to indicate the magnitude of the fluctuations in the respiratory rate.

PALLADIN (**19**), experimenting with etiolated shoots of *Vicia faba*, showed that sudden temperature changes as such acted as stimuli

[1] Abstract of thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany in the Graduate School of the University of Illinois, 1929.

upon the respiration rate. WOLKOFF and MAYER (24) had earlier noted that a sudden fall in temperature caused a distinct decrease in the respiratory rate below that normally found at the lower temperature, but a sudden rise had no effect. Gradual fluctuations about a mean had no apparent influence. DETMER (9), ZIEGENBEIN (25), KUIJPER (13), and BLANC (5) all failed to find a stimulation in the respiratory rate as the result of sudden temperature change. They believe the rate of respiration to change gradually with the temperature.

That the temperature of the roots affects the growth of the shoot has been conclusively shown by BIALOBLOCKI (3), BURKHOLDER (6), CANNON (7), and many others, particularly the observations of LINFORD (16), TISDALE (23), and other plant pathologists using the Wisconsin soil-temperature tanks in their studies of the influence of the environment upon the plant and its susceptibility to disease. In general, plants growing in a soil at moderately low temperatures have a larger root system and a coarser heavier shoot than do plants grown at the same air temperature but with a higher soil temperature. The effect of a temperature shock upon the roots in relation to the behavior and future development of the plant does not appear to have been investigated.

LEHENBAUER (14) has briefly but adequately reviewed the work on the effect of sudden temperature changes upon plant growth. It seems established that a sudden temperature change deranges the metabolism of the plant, resulting in either a depression or an acceleration of the growth rate, depending upon the direction and magnitude of the temperature change and also to some extent upon the period of exposure.

Material and methods

Seeds of *Phaseolus vulgaris* (Burpee's Stringless Greenpod) were carefully selected for soundness, and measured to secure uniformity of size. These beans were then germinated in clean quartz sand at 45 per cent of the water-holding capacity. At the end of the third day at 22° C., and before the secondary roots appeared, the seedlings were taken from the sand and their seed coats removed. The roots were carefully thrust through the holes in some no. 5 one-holed rub-

ber stoppers, which were supported over tap water by means of wooden frames. The container in which the seedlings were growing was covered with a larger one that provided ample ventilation and yet shut out the light. All of the seedlings were grown in tap water, which was frequently changed. When the plants were 6–7 days old and their hypocotyls had elongated sufficiently to extend through the stoppers, cotton was packed around them to hold them in posi-

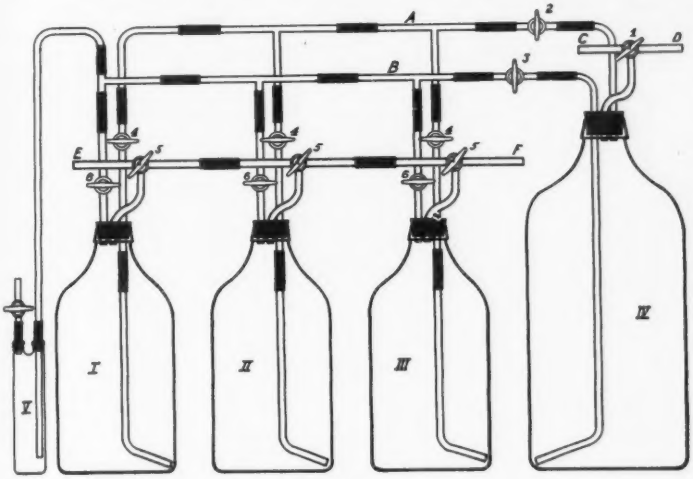


FIG. 1.—Gas collection apparatus

tion. The seedlings were left in this manner until they were needed for experimentation.

The aspirator and gas collection apparatus consisted of two sets of four bottles each, arranged as shown in fig. 1. A vacuum tank, exhausted by an automatically regulated electrically driven pump, maintained the uniform vacuum necessary for suction.

The gas collection apparatus was filled with a liquid which absorbed little or no carbon dioxide. The solution used was that of Layng and Crum, as reported by HOTTES and HAFENRICHTER (12). This liquid consists of a 35 per cent aqueous solution of zinc sulphate to which 14 gm. of concentrated sulphuric acid per liter of solution is added. Considerably more pressure or suction is needed to force

or draw this liquid through the apparatus than is required for water, but in spite of this difficulty it has proved very satisfactory.

Each of the bottles I, II, and III has a capacity of 4 liters, while the capacity of bottle IV is 12 liters. Stopcocks 2, 3, 4, and 6 all have a 2 mm. bore. It was found necessary to use stopcocks of this size when it was desired to aspirate at the rate of 3 or 4 liters in 10 minutes; at slower rates of aspiration stopcocks of 1 mm. bore should prove satisfactory. The three-way stopcocks, which serve for the passage of gas only, are of 1 mm. bore. Heavy pure gum rubber tubing was used on all joints, the rubber connections and stoppers being wired into place. Bottle V served as a safety valve.

To aspirate, stopcock 1 is turned to connect bottle IV with the vacuum through *D*, and stopcock 2 opened. Next stopcock 4 is opened on the bottle in which it is desired to collect the gas, in this case bottle I. The three-way cock 5 is then turned to connect the bottle with the respiratory chamber through *E*. The vacuum of 6 cm. now draws the liquid out of bottle I through tube *A* into bottle IV, and as the level of the liquid falls in bottle I the gas from the respiratory chamber is drawn into it. The speed of aspiration may be governed by the insertion of a piece of capillary tubing of the proper bore into tube *A*, between stopcock 2 and bottle IV. At the end of the period of gas collection stopcock 4 is closed and 5 turned to seal bottle I but to permit a through passage from *E* to *F*. For continuous gas collection, stopcocks 4 and 5 on bottle II should be opened before cocks 4 and 5 on bottle I are closed. With tight connections no solution can be withdrawn from bottle II until cock 5 on bottle I is turned to permit a through passage from *E*. If it is desired to have a period of aspiration between gas collections, it can easily be accomplished by attaching the vacuum, properly reduced, to *F*. In any case it is best to have the vacuum attached here at all times, to minimize the chances of inadvertently admitting air to the system by turning the wrong stopcock. The same procedure is then followed with bottles II and III.

To measure the volume of gas collected and to take a sample, the tube connecting the respiratory chamber with *E* is removed and replaced by one leading to the gas meter. The tube connecting with the vacuum at *F* must either be removed or the vacuum shut off.

Stopcock 2 is now closed and cock 1 turned to connect with the air pressure line through *C*. The air pressure was maintained at 150 gm. per square centimeter. Stopcock 3 is opened, followed by stopcock 6 on bottle I, and lastly cock 5 is turned to connect bottle I with the gas meter through *E*. As the liquid is now forced out of bottle IV into bottle I, the gas is forced out of bottle I through the meter. The sample was taken as the gas left the meter.

The gas meter used was one designed especially for respiration work, and was graduated to hundredths of a liter but permitted accurate estimates to thousandths. The samples were taken with the type of sampler described by BAILEY (1), and the gas analysis was made with BAILEY'S modification of the Haldane-Henderson gas apparatus. All gas volumes were reduced to the dry state at 0 °C. and 760 mm. mercury pressure. The weight of carbon dioxide in milligrams was obtained with the aid of the table of densities given by PARR and KING (20). Five place logarithms were used throughout.

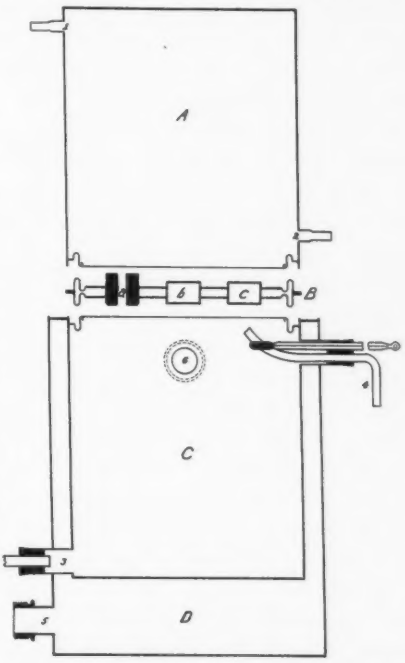


FIG. 2.—Respiration chamber: *A*, respiration chamber proper (1, 2, inlet and outlet respectively); *B*, double lid (*a*, *b*, *c*, brass tubes through lid and a rubber stopper inserted in *a*); *C*, inner root chamber (3, inlet and outlet tube; 4, overflow tube with thermometer inserted above); *D*, outer root chamber (5, 6, hose connections through which cooling liquid was circulated between *C* and *D*).

The respiration chamber is shown diagrammatically in fig. 2. It consisted of two parts, the respiration chamber proper (*A*), and the chamber in which the roots were held (*C*). Both were good quality

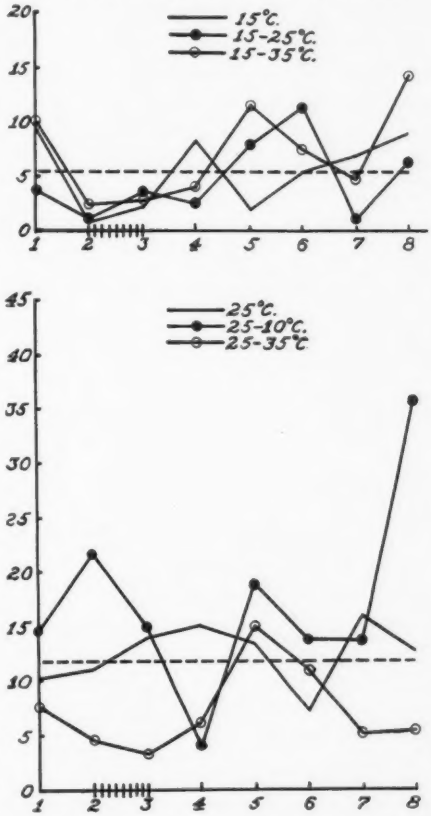
4-liter tin cylinders with tightly fitting pry lids. The lids were soldered together (*B*), seven holes punched through them, and short brass tubes soldered into these openings (*a*, *b*, etc.). The plants in their no. 5 stoppers were later forced into these tubes. The respiration chamber was thus inverted over the root chamber, which was soldered into a larger tin cylinder (*D*), making a double-walled chamber. Water or brine was circulated in the space between the two cylinders to control the temperature of the roots. An opening (3) was also provided in the root chamber, by means of which the water in which the roots were growing could be rapidly changed. The second opening in the root chamber carried an overflow tube (4) and a thermometer. The respiration chamber proper had two openings, an inlet tube (1) near the upper end of the cylinder and an outlet tube (2) near its lower extremity.

By means of this apparatus it was possible to increase or decrease the temperature of the water in which the roots were growing in about 6 minutes. During the entire test the respiration chamber was kept in one of the constant temperature cases which are standard equipment in the laboratory. The range of temperature fluctuation within the case was 1°. The air entering the respiration chamber was drawn through a Truog absorption tower filled with shell-caustic and then through a Winkler tube filled with 40 per cent potassium hydroxide.

When ready for an experiment, the seven selected seedlings in their rubber stoppers were forced into the holes in the double lid and the cotton removed from around the plants. Next the plants were sealed into the stoppers with chewing gum. Gum was the only seal found which did not injure the plants but would withstand the necessary vacuum. After the gum had set, the rim of the lid was lightly greased with stopcock lubricant and the respiration chamber proper forced into place. The lid was then loosened from the lower can and the chamber tested under 2.5 cm. vacuum for about 5 minutes. The respiration chamber was then placed in the temperature case and the connections with the aspirator and absorption tubes made.

The plants were always placed in the case at five o'clock of the afternoon before the test and a slow rate of aspiration maintained

all night. The actual test was begun either at seven or eight o'clock the following morning. At the end of the experiment the plants were removed from the chamber, any adhering gum washed off with alcohol, and the plants placed in the drier in preparation for the dry weight determination. All of the experiments were run in duplicate or triplicate, and the data are recorded on the basis of the dry weight of the seedlings at the end of the experiment. A total of 25 experiments, using seven seedlings each, were run. The values recorded in table I represent the averages of 194 carbon dioxide determinations and approximately 500 gas analyses.



FIGS. 3, 4.—Fig. 3 (above), respiration rates of seedlings of 15° C. group; fig. 4 (below), respiration rates of 25° C. group of seedlings; all values from table I (abscissae represent time in hours; cross-hatched portion indicates time roots were at second temperature; ordinates represent mg. CO_2 emitted per hour per gm. dry weight).

Experimentation

The data for each temperature series are presented in table I and figs. 3, 4, and 5. The dotted horizontal line through each graph represents the total average rate of respiration per hour per gram dry weight at the constant temperature of the group. These average rates of respiration are 5.34 mg. of carbon dioxide per hour at 15° C.,

11.43 mg. of carbon dioxide at 25° C., and 8.35 mg. of carbon dioxide per hour at 35° C. In those experiments in which the temperature of the water in which the roots were growing was changed, the change was made at the beginning of the third hour and the return to former temperature was at the beginning of the next hour. The cross-hatching of the abscissae indicates the period during which the roots of the plants were at the changed temperature. In the designation of any series in which two temperatures are used, the second temperature indicated always refers to the one to which the roots were changed during the third hour.

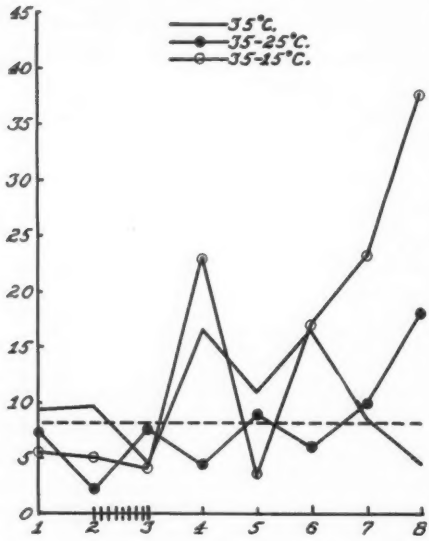


FIG. 5.—Respiration rates of seedlings of 35° C. group, all values from table I (abscissae represent time in hours; cross-hatched portion indicates time roots were at second temperature; ordinates represent mg. CO_2 emitted per hour per gm. dry weight).

15° C. GROUP.—The data for the 15° C. group are shown graphically in fig. 3. A striking feature is that all of the curves show a falling respiratory rate for the first 2 hours. The most logical explanation would appear to be that there had been a slight accumulation of carbon dioxide in the respiration chamber during the night, due to a slower aspiration rate; however, since the same rate was used throughout all of the experiments, one would expect the same type of declining curve at 25° and 35° C., but it is present in only one or two cases at these temperatures. The writer is unable to explain the coinciding of these curves. The 15° C. constant temperature curve is notable mainly for its rather wide fluctuations from the mean. The chief difference noticeable between the 15°–25° and 15°–

35° C. curves is that the former rises during the time that the roots are at the warmer temperature, while the latter remains at a uniform level. The shock of the change to 35° C. seems to have been severe enough to cause the respiratory process to remain on a level with that of the previous hour. The 15°–25° C. curve, on the other hand, rises during the third hour, probably because the temperature change is too small to depress the respiration rate for the entire hour. This curve shows a decrease in the respiration rate for the fourth hour, apparently due to the effect of the sudden change back to 15° C. The 15°–35° C. curve shows no signs of a second shock.

TABLE I

RESPIRATION RATES OF ETIOLATED BEAN SEEDLINGS, 7–9 DAYS OLD, WHEN SUBJECTED TO DIFFERENT TEMPERATURE TREATMENTS, IN MG. CARBON DIOXIDE PER HOUR PER GM. DRY WEIGHT

HOURS OF EXPERIMENTATION	15° C. GROUP			25° C. GROUP			35° C. GROUP		
	15°	15°–25°	15°–35°	25°	25°–10°	25°–35°	35°	35°–25°	35°–15°
1.....	9.48	3.58	10.26	10.19	14.29	7.59	9.28	7.84	5.37
2.....	0.77	1.05	2.47	10.93	21.67	4.64	9.68	2.25	5.04
3.....	2.02	**3.52***	**2.69***	13.92	**14.90***	**3.26***	4.58	**7.89***	**4.02***
4.....	8.13	2.56	4.17	15.09	4.03	6.07	16.44	4.23	23.15
5.....	1.97	7.97	11.56	13.08	18.90	14.94	11.03	8.73	3.31
6.....	5.44	11.65	7.52	7.16	13.66	10.93	16.51	6.07	16.74
7.....	7.05	0.89	4.77	15.82	13.66	5.04	8.48	10.02	23.35
8.....	9.02	6.33	14.49	12.67	35.74	5.42	4.31	18.06	37.56

* Hour at the changed temperature.

The difference between these two curves is more easily understood when it is remembered that 25° C. is optimum temperature for the bean plant.

25° C. GROUP.—Fig. 4 presents the averages for the 25° C. group. All of the curves for the experiments in which the temperature was changed show a fall during the third hour, which in the 25°–10° C. series is continued in the fourth hour. That the temperature change acted as a stimulus is indicated by the remarkable rise in the eighth hour of the 25°–10° C. series. Changing the roots from 25° to 35° C. flattened the curve between the second and third hour. The curve then rises, the maximum rise being during the fifth hour.

35° C. GROUP.—The greatest fluctuations in the respiration rate of the entire study are found in this group. Fig. 5 presents the curves

for the various series. The large stimulation shown by the 35°–15° C. curve is probably due to the 20° difference between the two temperatures used. Again the temperature change has flattened the curve for the hour at the changed temperature, and is followed immediately by a rapid rise. The 35°–25° C. curve indicates that this temperature change had little effect other than to depress the respiration rate for the four or five hours following the change. It is impossible to say that there was any shock effect in this case.

Discussion

These experiments indicate that sudden temperature changes upon the roots of etiolated bean seedlings produce a shock which is transmitted to the shoot, as evidenced by its changed respiratory rate. The magnitude of the shock does not appear to be in proportion to the difference between the two temperatures employed. This is brought out by a comparison of the curves for the 15°–35°, 25°–10°, and 35°–15° C. series. The 15°–35° and 35°–15° C. series received shocks of identical magnitude, yet the former shows comparatively little shock effect while the latter shows the greatest effect of the entire group of experiments. Further, the shock shown by the 25°–10° series is greater than that for the 15°–35° C. series. These facts seem to indicate that the direction of the shock is more important than its magnitude.

In a general way this conclusion is borne out by PALLADIN'S (19) results. He found an increase of 53 per cent in the amount of carbon dioxide given off over that of the control series, when the etiolated *Vicia faba* shoots floating on saccharose solution, which had been at a temperature of 36°–37.5° for several days, were tested at 18°–22° C. Similar shoots floating on saccharose solution transferred from 7°–12° to 18°–22° C. showed an increase of only 40 per cent in their carbon dioxide output. In this case the range of temperatures was also different, however, there being about 18° difference between the medium and high temperatures, and only about 9° C. difference between the medium and low temperatures. The response was certainly not in proportion to the temperature difference, but it is impossible to say definitely that the direction of the change influenced it.

A study of the graphs of the various series shows that the 25° curve has fewer sharp fluctuations than either the 15° or the 35° C. curves. The 25° plants were at a temperature which is very near their optimum temperature for growth. Apparently their metabolic processes were going on at a uniform rate, the equilibrium of which is hard to disturb. This was not true for the other two groups. These results are contrary to the work of most investigators, who have found that the rate of respiration is more uniform at lower temperatures than it is at medium or high temperatures.

The type of response to the temperature change also varies. Thus in the 15° group and the 25°–35° C. series the curves showing the respiration rate are comparatively flat from the second to the fourth hours. For the 25°–10° and the 35°–15° C. series the curves begin to fluctuate after the third hour instead of the fourth. These last two series also show the greatest range of fluctuations of the entire group of experiments, consequently the difference in type of response must also vary with the effectiveness of the stimulation.

The marked depression in the average respiratory rate at 35° C. seems largely due to the action of a "time factor." It must be remembered that the average respiratory rates, indicated by the dotted lines in figs. 3, 4, and 5, represent the rate of respiration after approximately 14 hours' exposure to the respective temperatures. The magnitude of the depression may be seen when the values of Q_{10} for the present data are compared with the results obtained by KUIJPER (13) for pea seedlings, and by LUNDEGARDH (17) for the leaves of the potato plant. KUIJPER's respiration results are based on the first hour at these temperatures, while those of LUNDEGARDH cover a period 5–17 minutes. These results are:

Temperature	15° C.	25° C.	35° C.
Q_{10} of present data	2.14	0.70	
Q_{10} of KUIJPER's data	2.34	1.58	
Q_{10} of LUNDEGARDH's data	2.20	1.30	

Plainly the value of Q_{10} for the 10° between 25° and 35° C. in the present experiments is much lower than in the other two cited.

The present experiments also indicate clearly the close connection between the activities of the shoot and those of the root. By just what means a shock administered to the cells of the root is trans-

mitted to the cells of the shoot is impossible to say at present. SNOW (21) and others have advanced the idea that a hormone or similar substance is formed as the result of a stimulus, and is then carried to all parts of the plant. Others believe that the stimulus is of an electrical nature, and passes from cell to cell by means of the slender connecting strands of protoplasm or plasmodesma.

Regardless of the method of conduction, it is certain that in this case a stimulus acting upon the root has affected the metabolism of the shoot. From this it might be concluded that a stimulus acting upon one part of the plant will influence the entire plant. That this is not always the case is illustrated by the various treatments used to break up the rest period of woody plants, in which only the portion of the plant actually treated has its rest period broken. Similarly, GARNER and ALLARD (11) have found localized response to length of day in cosmos. It is realized that these cases are not exactly comparable with the present experiments, for here we are dealing with the effect of a stimulus upon a portion of a plant which could be separated from the remainder of the organism without seriously injuring it. It seems, therefore, that the effect of a stimulus acting upon the entire root or shoot is different in respect to transmission from the effect if it had acted upon only a small portion of the plant.

The action of the stimulus may perhaps best be understood by a consideration of the cell and its metabolism. A number of investigators consider the cell as a colloidal complex in which the various chemical reactions proceed. SPOEHR (22) has expressed this concept as follows:

> It seems highly improbable that the life processes consist of any one series of chemical changes or are dependent upon any particular molecule or chemical group. But rather the simple life processes entailing energy changes may perhaps be regarded as a complex of interrelated chemical changes taking place in a certain medium or substratum. This medium, colloidal dispersion, mixture, or aggregate of various substances is the seat or substratum in which the various chemical reactions take place, the nature and course of which are determined by the complex of properties associated with water control, surface phenomena, and of course catalysts (such as inorganic salts and enzymes). These colloids do not enter into or support the chemical reactions or do so probably only in a rather indirect manner, serving primarily as a physical medium. Such a system would be of a heterogeneous nature and, of course, of the most complex type,

and capable of various adsorption phenomena productive of localized action which would influence the function as well as the structure of the system. In fact, such a hypothesis would demand that the substratum be relatively stable, that the colloidal material once formed does not break down as readily as the other substances, or only after the supply of these has been exhausted. This does not mean actual or chemical stability, but rather relative to the other substances under the existing physiological conditions, as, for instance, relatively slightly dissociated by salts or other catalysts and resistant to the action of enzymes.

Plant respiration is at present conceived to be a complicated life process which takes the form of a chain of catalytic reactions in which the products formed by one reaction become the reactants in the next link. These reactions, in general, follow the law of mass action which in its simplest form states that the rate at which any reaction proceeds is directly proportional to the amount, or rather the concentration, of the reacting substance. In other words, any change in the amount of one of the reactants brings about the establishment of an entirely new equilibrium, with a resulting increase or decrease in the amount of end product. Further, there is the probability that the individual steps may respond differently to external influences, for example, they might have different temperature coefficients, in which case a rise in temperature might accelerate one link more than the others, resulting in an excess of its end product; or a lowering of the temperature might retard one reaction more than the others. This reaction would then become the limiting factor in the series.

Does the stimulus of these experiments act directly upon this chain of reactions by changing the speed of one or more links, or does it act indirectly by first influencing the structure of the protoplasm? These two viewpoints are not necessarily opposed. Enzymes are very sensitive to temperature, and it is entirely probable that the temperature change has affected the rate of enzyme activity directly, thus changing the speed of one or more links in the chain of reactions. On the other hand, a number of modern workers incline to the view that protoplasm has a definite structure, and that the reactions proceed at the interphase boundaries. LILLIE (15) states:

Since the formation of films is a characteristic feature of the processes at phase boundaries in polyphasic systems of all kinds, and especially in protoplasm, it is probable that films are present at all sharply defined boundary

surfaces in the living cell, i.e., not only at the general surfaces of the cell and of cell structures like nuclei and vacuoles, but also at the surfaces of fibrils, granules, chromosomes, mitochondria, the Golgi apparatus, and other structures. Variations in the physical or chemical properties of these films will influence the catalytic and other properties of the surfaces, and hence the nature and rate of the chemical reactions occurring in the cell under the surface influence. Electrical factors are of special importance in all such effects; . . . . Since these films are readily broken down and reformed under often slight changes of condition, the state of the protoplasmic surface layers is subject to continual change; the rate and character of the chemical reactions occurring under the influence of these surfaces are affected correspondingly. There are many indications (apart from the action of narcotics) that the special sensitivity of the protoplasmic system to chemical, electrical, and other changes of condition, i.e., its irritability, is to be referred chiefly to this variability of the protoplasmic films.

It seems clear that any change in the rate of one of the steps in the chain of linked reactions, as the result of a stimulus, would influence the entire chain and consequently result in a changed amount of carbon dioxide being released. This explanation will satisfactorily explain the change in respiratory rate upon a change in temperature, but it will not explain the constant fluctuations in the respiratory rate just noted. The continual breaking down and building up of protoplasmic films, with the resulting changes in surface action, would seem to offer a more probable explanation for these fluctuations. Probably both of these processes are concerned. Protoplasm undoubtedly has a definite structure, for the mere presence of the chemical and physical components usually found in the cell does not produce a living cell. Further, when the structure of the cell is destroyed, profound physical and chemical changes at once appear. On the other hand, many of the chemical reactions proceeding within the cell have already been established. Thus both explanations have a sound basis in fact.

Summary

1. A definite stimulation of the respiratory rate of the shoot is shown to occur as a result of temperature change of the root.

2. The amount and type of stimulation vary with the temperature at which the plants are held and the direction of the temperature change.

3. The magnitude of the stimulation does not appear to be defi-

nitely related to the size of the difference between the two temperatures used.

4. The 25° C. constant temperature series shows smaller fluctuations from its mean respiratory rate than does either the 15° or the 35° C. constant temperature series.

5. A study of the temperature coefficients shows that a marked depression in the respiratory rate occurred at 35° C., probably due to a "time factor."

6. A new type of aspirator and gas collection apparatus is described.

The writer wishes to acknowledge his indebtedness to Professor CHARLES F. HOTTES, under whose direction the investigation was undertaken, for his aid in the construction of apparatus and for his most helpful criticism and suggestions during the progress of the work.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

[*Accepted for publication March 7, 1930*]

LITERATURE CITED

1. (BAILEY, C. V.,) HAWK, P. B., and BERGEIM, O., Practical physiological chemistry. pp. 491–529. P. Blakiston's Son & Co. Philadelphia. 1926.
2. BENNETT, J. P., and BARTHOLOMEW, E. T., The respiration of potato tubers in relation to the occurrence of blackheart. Univ. Calif. Agric. Exp. Sta. Tech. Paper 14. 1924.
3. BIALOBLOCKI, J., Über den Einfluss der Bodenwärme auf die Entwicklung einiger Culturpflanzen. Landw. Ver. Sta. **13**:424–472. 1871.
4. BLACKMAN, F. F., and PARIJA, P., Analytic studies in plant respiration. I. The respiration of a population of senescent ripening apples. Proc. Roy. Soc. London B. **103**:412–445. 1928.
5. BLANC, L., L'influence des Variations de Température sur la Respiration des Plantes. Rev. Gen. Bot. **28**:65–79. 1916.
6. BURKHOLDER, W. H., The effect of two soil temperatures on the yield and water relation of healthy and diseased bean plants. Ecology **1**:113–123. 1920.
7. CANNON, W. A., and FREE, E. E., Physiological features of roots. Carnegie Inst. Wash. Publ. 368. 1925.
8. CZAPEK, F., Biochemie der Pflanzen. Bd. III. G. Fischer. Jena. 1921.

9. DETMER, W., Beobachtungen über die normale Athmung der Pflanzen. Ber. Deutsch. Bot. Ges. **10**:535–539. 1892.
10. FERNANDES, D. S., Aerobe und anaerobe Atmung bei Keimlingen von *Pisum sativum*. Récueil Trav. Bot. Néerl. **20**:170–256. 1923.
11. GARNER, W. W., and ALLARD, H. A., Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agric. Res. **23**:871–920. 1923.
12. HOTTES C. F., and HAFENRICHTER, A. L., A constant rate aspirator. Science N. S. **67**:320–322. 1928.
13. KUIJPER, J., Über den Einfluss der Temperature auf die Atmung der höheren Pflanzen. Récueil Trav. Bot. Néerl. **7**:130–240. 1910.
14. LEHENBAUER, P. A., Growth of maize seedlings in relation to temperature. Phys. Res. **1**:247–288. 1914.
15. LILLIE, R. S., Reactivity of the cell. Section IV. 165–234. General cytology, edited by E. V. COWDRY. Univ. Chicago Press. Chicago. 1924.
16. LINFORD, M. B., A *Fusarium* wilt of peas in Wisconsin. Univ. Wis. Agric. Exp. Sta. Res. Bull. 85. 1928.
17. LUNDEGARDH, H., Der Temperaturfaktor bei Kohlensäureassimilation und Atmung. Biochem. Zeitsch. **154**:195–234. 1924.
18. MATTHAEI, G. L. C., Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon-dioxide assimilation. Trans. Roy. Soc. London B. **197**:47–105. 1904.
19. PALLADIN, W., Influence des changements de température sur la respiration des plantes. Rev. Gen. Bot. **11**:241–257. 1899.
20. PARR, S. W., and KING, W. R., The density of carbon dioxide with a table of recalculated values. Univ. Ill. Eng. Expt. Sta. Cir. 13. 1926.
21. SNOW, R., Transmission of stimuli in plants. Nature **115**:82. 1925.
22. SPOEHR, H. A., The carbohydrate economy of cacti. Carnegie Inst. Wash. Publ. 287. 1919.
23. TISDALE, W. B., Influence of soil temperature and soil moisture upon the *Fusarium* disease in cabbage seedlings. Jour. Agric. Res. **24**:55–86. 1923.
24. WOLKOFF, A., and MAYER, A., Beiträge zur Lehre über die Athmung der Pflanzen. Landw. Jahrb. **3**:481–527. 1874.
25. ZIEGENBEIN, E., Untersuchungen über den Stoffwechsel und die Athmung keimender Kartoffelknollen sowie anderer Pflanzen. Jahrb. Wiss. Bot. **25**:563–606. 1893.

EFFECT OF MANGANESE, COPPER, AND ZINC ON GROWTH AND METABOLISM OF ASPERGILLUS FLAVUS AND RHIZOPUS NIGRICANS[1]

J. S. McHARGUE AND R. K. CALFEE

(WITH SEVEN FIGURES)

Fungi absorb mineral nutrients, such as manganese, copper, or zinc, from the soil or from solutions, but not possessing photosynthetic metabolism, they are dependent upon plant or animal products for their food. In the natural growth of fungi, therefore, manganese, copper, and zinc in small amounts are present in the medium on which they grow. In a synthetic medium such as the average laboratory culture, these elements usually are present as impurities in sufficient quantities for the normal growth of plants.

The findings of previous investigators, although somewhat inconsistent, seem to indicate that manganese, copper, and zinc are necessary for the normal growth of such species of fungi as have been investigated. SAUTON (5) concluded from his experiments that manganese was as indispensable to the spore formation of *Aspergillus fumigatus* as was oxygen. WATERMAN (8) found that manganese increased the rapidity of spore formation. He also investigated the effect of copper and zinc, finding that certain concentrations of zinc sulphate, zinc chloride, and copper sulphate increased the weight of his cultures but decreased spore formation proportionately. WATERMAN considered the influence of copper and zinc unfavorable. Zinc was found by LEPIERRE (3) to exert, under certain conditions, a favorable influence on the growth of *A. niger*, but not to be absolutely essential for complete development. JAVILLIER (2) observed that zinc in a concentration of one part to two million parts of medium increased the volume of growth of *A. niger* 58 times. Further work

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

by JAVILLIER showed that zinc could not be replaced by cadmium or beryllium. STEINBERG (6) obtained a minimum growth with *A. niger* in the absence of iron and zinc, and regarded both metals as essential for normal growth. Later experiments by STEINBERG indicated that iron and zinc could partly be replaced by uranium or cobalt. Metallic zinc was found by BORNAND (1) to decrease the growth of *A. niger*. ROBERG (4) studied the effect of pure compounds of iron, copper, and zinc on the growth of representative species of *Aspergillus*. Iron and zinc were shown to be essential for growth but the results obtained with copper were not decisive. The writers believe the indecisive result obtained with copper was due to his inability to exclude copper contamination in the nutrient solution.

To ascertain the effects of manganese, copper, and zinc on the growth and metabolism of molds, it was necessary to prepare a medium favorable for their development but free from these elements. A solution containing 1 per cent ammonium sulphate, 0.5 per cent monopotassium phosphate, 0.4 per cent potassium sulphate, 0.25 per cent magnesium sulphate, 0.25 per cent calcium sulphate, and 5 per cent glucose in distilled water produced a heavy, rapid growth of several molds. A standard medium of this composition, using sucrose instead of glucose (because the latter contained impurities) was then prepared, employing only salts that had been shown to be free from manganese, copper, and zinc by chemical tests. The distilled water used in all the cultures was prepared by means of a quartz tube condenser. In purifying these salts from manganese, copper, and zinc, other elements usually present as impurities were also removed. To avoid any influence due to the absence of these elements, 0.001 per cent ferric citrate, 0.0001 per cent potassium iodide, and 0.01 per cent potassium chloride were included in the medium. Agar could not be obtained free from manganese, zinc, and copper, so silica gel was added to the liquid medium for solid cultures.

Growth of molds on this medium was very weak and slow. A green *Aspergillus* of the *flavus* group made the most vigorous growth, and was isolated in pure culture. Six transfers of spores were made on the standard medium to prevent any appreciable amount of manganese, copper, and zinc being carried over in the inoculum, before

the experimental culture was accepted. Cultures were incubated at different temperatures, and optimum growth was obtained at 28° C.

Cultures were then started in three series of flasks, using 100 cc. of medium in each. Manganese sulphate was included in the medium in the flasks of the first series in such quantities that the manganese content of each flask differed five parts per million from that of each adjacent flask. The range in concentration covered by the series was from a blank control (0) to 30 parts per million. Copper sulphate in corresponding amounts was added to the second series of flasks, and in the third series zinc sulphate was varied by five parts of zinc per million from a blank to 30 parts per million. Each of the salts of manganese, copper, and zinc added to the medium was known to be free from the other two elements. Growth was rapid in some flasks, a maximum being reached in five days. Cultures were then filtered and the fungi washed with distilled water to remove adhering medium. An excess of water was used over the amount found necessary to remove sulphates, and the same quantity was used in washing each culture. The weight of each culture was recorded after drying at 100° C. to constant weight, and compared with the controls and with each other as the amounts produced in 100 cc. of medium in five days.

All cultures containing manganese, copper, or zinc produced a greater weight than the controls (fig. 1). The greatest weight in the manganese series was produced by the culture containing five parts per million of this element. A nearly uniform decrease in weight occurred with each addition of manganese over this concentration, up to 25 parts per million. Thirty parts per million produced a slightly greater decrease than 25 parts per million. The conidia were a yellowish green when manganese was present, and a pale green to white in its absence. At 20 parts per million manganese appeared to be toxic to the *Aspergillus*, and conidiophores were not produced abundantly, none being produced near the edges of the colony. Twenty-five parts per million and greater concentrations were decidedly toxic, the mycelium being curled and conidiophore production greatly retarded. The optimum concentration of manganese sulphate was below five parts per million.

The series of cultures containing copper also produced a maximum

growth at a concentration of five parts per million, and lost weight with each increase in the copper content. The increase in weight with copper was not quite so great as that produced by manganese. Copper sulphate was toxic in concentration exceeding 15 parts per million. The spores of all cultures grown in the presence of copper had a uniform blue-green color. Zinc salts were toxic at a concentration of five parts per million. Conidia were not so abundant as in the

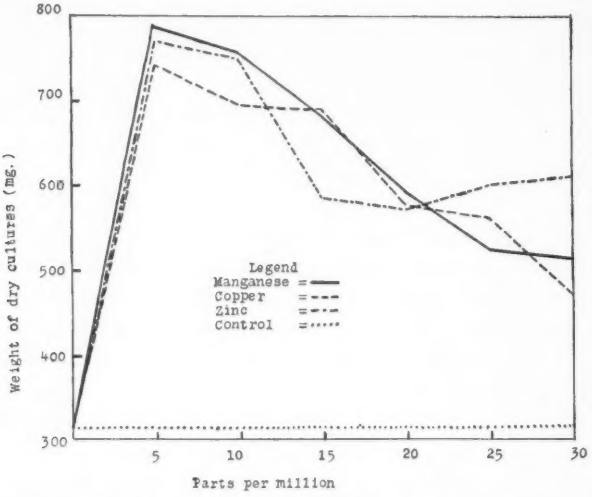


FIG. 1.—Amounts of growth produced in cultures containing varying amounts of manganese, copper, and zinc.

series containing manganese or copper. The colonies were wrinkled and curled, and marked by white edges and light zones. The greatest weight was produced in the presence of five parts per million of zinc. The weight of cultures decreased with increases in the concentration of zinc to 20 parts per million. Greater concentrations gave inconsistent results. The maximum weight produced in the zinc series was slightly greater than that of the copper series, but was exceeded by the maximum weight obtained in the manganese series.

The optimum concentrations of these salts were investigated on silica gel cultures, since it was evident that they were a factor in the

growth of the *Aspergillus*. These were found to be 5 parts per million for copper, 2.5 parts per million for manganese, and 1 part per million for zinc (fig. 2). Copper was also found to be more toxic when either

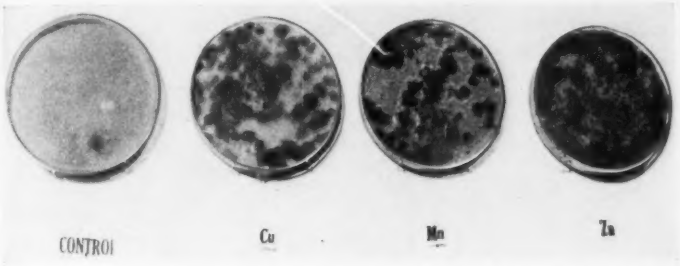


FIG. 2.—Sand cultures of *Aspergillus* 10 days old, showing stimulation of copper (5 ppm), manganese (2.5 ppm), and zinc (1 ppm) in optimum concentrations on growth.

manganese or zinc was present. The influence of the optimum concentrations of these metals was ascertained in fluid cultures of 250 cc. each. Manganese, copper, and zinc, and all combinations of these elements in the optimum concentrations were represented, otherwise the chemical composition of the cultures was identical. Such factors as length of time and temperature of sterilization, hydrogen-ion concentration, and temperature and period of incubation were the same for all cultures. The amount of spores used for inoculation probably varied in different cultures. Because of an oil which covered the spore, a suspension could not

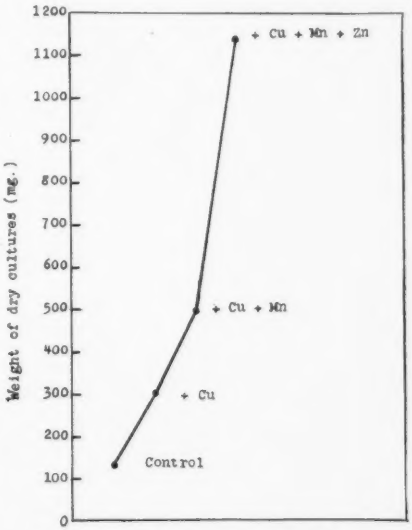


FIG. 3.—Effect of manganese and zinc added to copper.

be formed, so the smallest amount of material that could be handled on a pointed needle was transferred to each flask. All cultures were incubated at 28° for 5 days.

After attaining the optimum growth, the cultures were filtered, washed free from the nutrient solution, dried at 100 °C. to constant weight, and the weights determined and compared. Manganese and zinc influenced growth to a greater extent than did copper. In combination also they produced a greater effect than either metal in combination with copper. Fig. 3 shows the increase produced in total weight by copper over the control. The addition of manganese in the culture resulted in a greater increase, while the most rapid growth occurred when all three of these elements were in the culture. Manganese (fig. 4) produced an increase slightly greater than did copper. Manganese and zinc increased the weight to a much greater extent than did manganese and copper, or zinc and copper (fig. 5). Zinc with copper influenced growth to a greater extent than manganese and copper. The relation between these cultures, in terms of the control as 100 per cent, was:

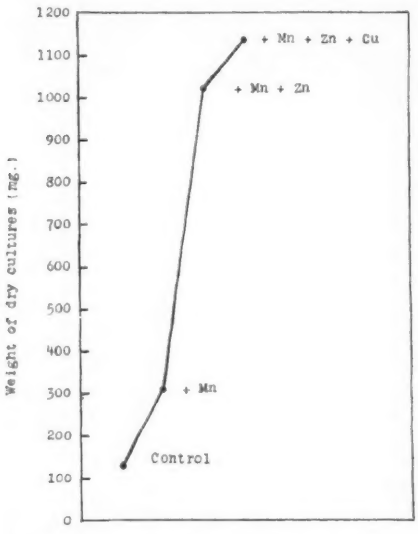


FIG. 4.—Effect of zinc and copper added to manganese.

CULTURE	PER CENT
Control	100.0
Copper	234.5
Zinc	241.0
Manganese	247.5
Copper+manganese	399.7
Copper+zinc	510.8
Manganese+zinc	857.9
Copper+manganese+zinc	900.6

The size of the colonies on silica gel plates compared closely with the weights of the dried mold from the liquid cultures. All plates containing copper, manganese, or zinc produced colonies of greater diameter than did the control plates. The growth was measured daily and recorded (fig. 6). The cultures were also photographed. Because of the greater contrast of the colonies (fig. 7), *Rhizopus nigricans* showed the effects of copper, manganese, and zinc more clearly when photographed than did the *Aspergillus*.

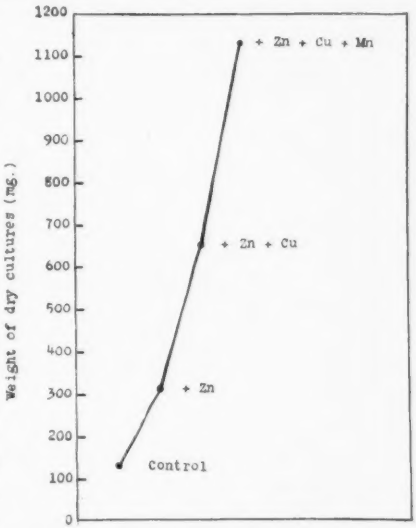


FIG. 5.—Effect of copper and manganese added to zinc.

The smaller volume of medium and the less rapid removal of catabolic products resulted in slower growth on silica gel for all cultures, as compared with that in liquid media. This effect was more pronounced on cultures making a large amount of growth. The control culture (fig. 6) made most of its growth between the first and third day after inoculation, and ceased growing on the fifth day. When manganese, copper, or zinc was present, a greater and more rapid growth was obtained, the growth curves for these elements corresponding closely to one another. Combinations of two of these elements also formed curves that were closer to one another than to the control curve, or to the curves of the elements when but one was present, or when all three were present. The sudden stopping of growth in the control culture indicated a deficiency of necessary nutrient elements rather than toxic inhibition, in view of the much greater and more rapid growth of the other cultures. This is also shown to a less marked extent by all cultures not having all three elements present. The decrease in the rate of growth of cultures con-

taining copper, manganese, and zinc might be due to the absence of other elements necessary for growth, or to toxic accumulations.

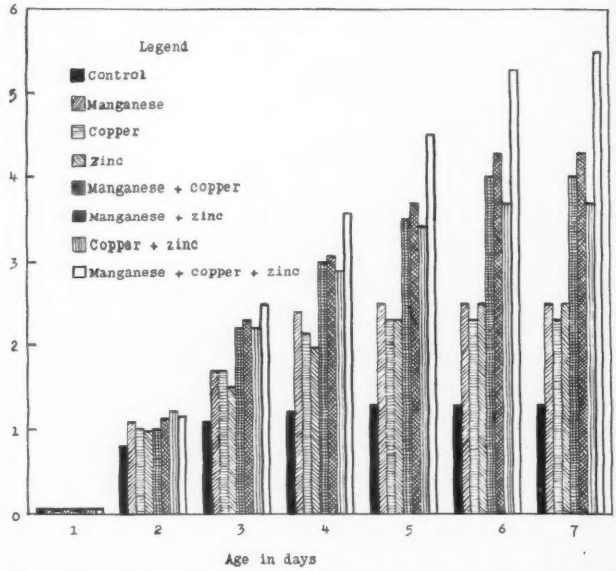


FIG. 6.—Effect of manganese, copper, and zinc on cultures grown on silica gel

FIG. 7.—Silica gel cultures of *Rhizopus nigricans* (upper row) and *Aspergillus* (lower row), fifth day of growth, showing influence of manganese, copper, and zinc, and their combinations on growth.

The presence of copper in the media apparently slightly increased the plants' ability to assimilate other mineral nutrients; manganese produced the opposite effect. The cultures grown on media containing manganese had lower percentages of ash than the control unless zinc was also present. All cultures containing zinc had a higher ash content than the control.

Copper was present in the ash of all cultures in greater quantities than either manganese or zinc, the percentage of copper being higher

TABLE I

MINERAL, NITROGEN, AND FAT CONTENT OF ASPERGILLUS IN PERCENTAGE OF MOISTURE-FREE MATERIAL

CULTURE	CONTROL	Cu 5 PPM	Mn 2.5 PPM	Zn 1 PPM	Cu 5, Mn 2.5 PPM	Cu 5, Zn 1 PPM	Mn 2.5, Zn 1 PPM	Cu 5, Mn 2.5, Zn 1 PPM
Weight of culture, as percentage of control	100	234.5	247.5	241.0	399.7	510.8	857.9	900.6
Ash	5.0	5.29	4.75	6.03	3.98	7.67	6.046	5.5
Copper	None	0.017	None	None	0.013	0.022	None	0.02
Manganese	None	None	0.0025	None	0.0025	None	0.0023	0.003
Zinc	None	None	None	0.009	None	0.0076	0.0097	0.0075
Phosphorus	0.9	0.977	0.157	1.26	0.95	1.14	1.415	0.957
Calcium	0.21	0.233	0.12	0.484	0.252	0.667	0.583	0.415
Magnesium	0.255	0.197	0.13	0.335	0.282	0.275	0.203	0.467
Iron	0.002	0.009	0.005	0.007	0.014	0.02	0.0076	0.008
Nitrogen	4.82	4.75	4.65	4.5	4.09	4.25	4.4	4.48
Protein (Nx 6.25)	30.1	29.75	29.00	28.1	25.6	26.6	27.48	28.00
Ether extract	1.64	2.03	2.55	2.25	2.51	2.57	3.355	3.5

in the presence of zinc and lower in the presence of manganese. The amounts of manganese in the ash varied but slightly in the presence of the other two metals. The zinc content was increased by manganese and reduced by copper.

Phosphorus, calcium, and magnesium were assimilated in larger quantities by cultures containing zinc. The absorption of these elements was lowered by the presence of manganese unless copper or zinc was present. Less magnesium was absorbed by the copper culture than by the control. The ether extract increased as growth increased, the increase being greater when manganese was present. Zinc was next to manganese in influencing the synthesis of the ether-soluble products.

The nitrogen content was less than the control in all cultures containing manganese, copper, and zinc. The percentages of nitrogen did not appear to be decreased by any one element, or uniformly decreased by an increase in growth. Apparently nitrogen decreased as growth increased, unless manganese and zinc were present. Analyses are given in table I.

No consistent differences could be found by microscopical measurements of the morphological structures of these cultures. The sizes varied in all cultures within the limits described by THOM and CHURCH (7) for the species.

Summary

1. Cultures of *Aspergillus* made a heavier, more rapid growth in a medium containing certain concentrations of manganese, copper, or zinc than in a medium free from these metals.

2. The optimum concentrations of these metals were low, and slightly larger quantities became toxic.

3. Combinations of the optimum concentrations of manganese, copper, and zinc stimulated a greater growth than did any one of the three metals, and all three produced a greater growth than did the combination of any two of these metals.

4. Manganese, copper, and zinc in the medium influenced slightly the assimilation of phosphorus, magnesium, and calcium.

5. The nitrogen content of *Aspergillus* grown in media containing manganese, copper, and zinc, or combinations of these elements, was less than in control cultures.

6. The fat content (ether extract) of *Aspergillus* was increased by manganese, copper, and zinc.

KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KY.

[*Accepted for publication March 21, 1930*]

LITERATURE CITED

1. BORNAND, M., Influence des métaux sur le développment de l'*Aspergillus niger* cultivé sur liquide de Raulin. Centbl. Bakt. **39**:488–496. Abs. Experiment Station Record 1914. **30**:824. 1913.

2. JAVILLIER, M., Recherches sur la substitution au zinc de diverses éléments chimiques pour la culture de l'aspergillus niger. Ann. Inst. Pasteur **27**:1021–1038. 1913.

3. LEPIERRE C., Inutilité du zinc pour la culture de l'*Aspergillus niger*. Compt. Rend. Acad. Sci. (Paris) 157. **2**:877–879. 1913.
4. ROBERG, MAX, Über die Wirkung von Eisen, Zinc und Kupfersalzen auf Aspergillen. Centbl. Bakt. **74**:333–370. 1928.
5. SAUTON, B., The spore formation of *Aspergillus fumigatus*. Compt. Rend. Soc. Biol. (Paris) **74**:38–39. Abs. Experiment Station Record 1913. **29**:30. 1913.
6. STEINBERG, R. A., Effect of zinc and iron compared with that of uranium and cobalt on growth of *Aspergillus*. BOT. GAZ. **70**:465–468. 1920.
7. THOM, C., and CHURCH, M. B., The Aspergilli. Williams and Wilkins Co. Baltimore. 1926.
8. WATERMAN, H. J., The action of potassium, sulfur, and magnesium on the metabolism of *Aspergillus niger*. K. Akad. Wetensch. Amsterdam. Proc. Sect. Sci. **15**:753–764. Chemical Abstracts **18**:1914. 943. 1912.

NUCLEAR DIVISION AND DEVELOPMENT OF STERIGMATA IN COPRINUS ATRAMENTARIUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 413

MARGARET MARTIN VOKES

(WITH FORTY-EIGHT FIGURES)

Introduction

The hymenia of the Basidiomycetes have been investigated by HOFFMAN (14), DE BARY (8), ATKINSON (1–3), and others. NICHOLS (23) and LEVINE (19) germinated individual spores in order to study the mycelia and sporophores at will. Cytologically, however, little has been done with this group, and this study was undertaken to consider that phase.

In 1884 STRASBURGER (28) studied the division of nuclei in the basidia of the Basidiomycetes. He stated that the nucleus divided into as many as eight, but that formation of the sterigmata began when the nucleus within the basidium had divided into two. He was certain centrospheres were present, either inside or outside the nucleus. In 1866 DE BARY (8) had observed a clear central portion in the basidia of the Agaricaceae, which he assumed to be a nucleus but which he said needed further study. ROSENVINGE (26), working with STRASBURGER, concluded that all the cells of the Hymenomycetes contained one or more nuclei. In the young basidia the single nucleus divided, forming four or eight nuclei, which, together with the protoplasm of the basidium, pushed their way up through the narrow neck of the sterigmata and into the spores. The number of nuclei was constant for the same species but not always for the genus.

Later WAGER (30–33) investigated the Hymenomycetes cytologically, chiefly *Agaricus stercorarius* and *Amanita muscaria*. He found the young basidia to contain two nuclei, together with a small amount of protoplasm and one or two vacuoles. At an early stage these nuclei fused, forming one large nucleus at the center of the basidium. This nucleus later took up a position near the apex of the

basidium, where during division a spindle appeared, and at each pole a deeply staining granule or centrosome was present. The resulting nuclei again divided. The nucleolus apparently played an important part during division, being found at the upper end of the nucleus. The daughter nuclei were similar to the parent nucleus, but smaller. Previously or during formation of the sterigmata the four nuclei became closely grouped at the base of the basidium, appearing almost fused. Spores protruded from the tips of the sterigmata, and protoplasm from the basidium passed into the spores. Not until then did the nuclei pass to the apex of the basidium, becoming so small that they could pass without difficulty into the spores, but whether such passage took place was not determined. WAGER (**31**) agreed with ROSEN (**25**), who studied *Lepiota* (*Armillaria*) *mucida*, that a much coiled thread was present within the network of the single nucleus.

DANGEARD (**4–7**) reviewed previous work, and after studying a number of species, concluded that all cells contained two nuclei, each having two chromosomes, and that these nuclei fused to form a single large nucleus in the basidium. Such fusion constituted a gametic union.

RUHLAND (**27**) investigated several species and found spindle rays as well as centrosomes and astral rays. HARPER (**13**) found spindles in *Hypochnus subtilis* on which were 6–8 chromosomes. That the binucleate condition existed in all cells of the sporophore was again definitely concluded. PETRI (**24**) observed, in *Hydnangium carneum*, strands which he considered extensions of the nuclear membrane and which connected the nucleus and the sterigmata.

One or more species in nearly every family of the Basidiomycetes was studied by MAIRE (**20–22**), and in all of them two nuclei only were observed to be associated with mitotic conjugation in the young basidia. The two nuclei fused, the chromosomes became thick, divided in two longitudinally, were torn apart, and went to the poles where astral rays were then emitted. The two nuclei formed produced spindles almost immediately, each having centrosomes and asters. Later four centrosomes occupied the extremities, and seemed to determine the location of the four spores. Before each centrosome went toward the apex of the basidium, a kinoplastic differentiation

was produced in the cytoplasm of the basidium; under the influence of this the nuclei were placed in a line along the axis of the basidium, and little by little were attracted toward the summit of the cell. The extremities of the sterigmata then swelled, and all the cytoplasm passed into the spores whose membranes had begun to enlarge at the time the nuclei reached the entrance of the sterigmata below. Two chromosomes were thought to be present in all species.

VAN BAMBEKE (**29**) reported fibers which connected nuclei and centrosomes in the basidia of *Hydnangium carneum*. Later FRIES (**10**) reported the same for *Nidularia pisiformis*. He found centrosomes and astral rays on the primary spindle, and longitudinal splitting of the spindle was thought to occur. GILBERT (**11, 12**) found centrosomes in *Dacryomyces* sp., and a dense staining body on the nuclear membrane during late synapsis which he believed gave rise to four centrosomes.

LEVINE (**17–19**), studying chiefly *Boletus* and *Polyporus*, found the two basidial nuclei fused. Spireme strands suggested splitting, and a shortening and segmentation of chromatin followed. The orientations of the spindle axes varied but were commonly transverse to the long axis. The polar asters of LEVINE (**18**) corresponded with those seen by MAIRE (**22**). The four basidial nuclei did not at any time go to the base of the cell and fuse, as WAGER had reported, but remained in the central position. At this time a spore initial in the form of a globular mass of cytoplasm became differentiated at the apex of each sterigma. A fibrillar strand was stretched from each centrosome at the apex of the sterigma to the nucleus, which was still in the basidium. This had been reported by MAIRE (**22**), who thought this strand contracted and pulled the nucleus up into the spore. In the species of *Boletus* studied there were 6–8 chromosomes, and although the number in the second division could not be determined, it was always more than two.

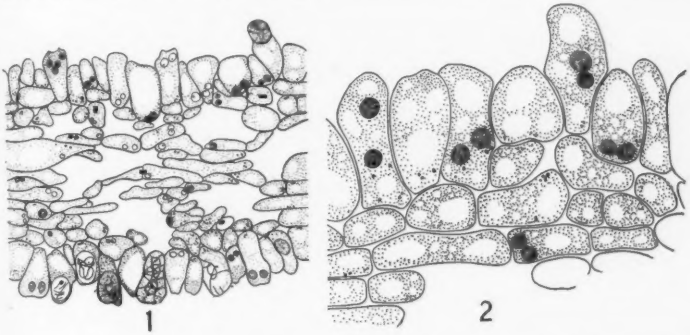
Eocronartium muscicola was studied by FITZPATRICK (**9**). His plates show spindles but do not show centrosomes. KUHNER (**15, 16**) found both centrosomes and spindles, and although division of the conjugate nucleus was thought to have taken place transversely, it usually occurred at the apex of the basidium.

Materials and methods

Several sporophores of *Coprinus atramentarius* were collected on the campus of the University of Chicago in October, 1928, by Professor C. J. CHAMBERLAIN. These sporophores were in the button stage and were cut in pieces 1–3 mm. cube and fixed in chromo-acetic-osmic solution. The paraffin ribbons were cut at 1.5 μ, and were stained with iron-alum-haematoxylin.

Investigation

Longitudinal sections of gills show loose arrangement of vegetative hyphae, the trama, through the center. The cells are binucleate



FIGS. 1, 2.*—Fig. 1, longitudinal section of young gill; fig. 2, early binucleate stage of hymenial layer.

* Figs. 1–3 were made with a Spencer fluorite objective 1.8 mm., combined with a Zeiss compensating ocular 10×. All figures were made with the aid of a Spencer camera lucida.

and hemispherical pads are present (fig. 1). Larger and slightly more compact cells, the hymenial layer, grow out from the trama, and here the basidial cells are produced which in turn produce the basidiospores (fig. 2). All have the contour of the basidia, but some are smaller and sterile (paraphyses). They are arranged more or less alternately with the spore-producing cells. Because of these paraphyses the spores are better able to expand.

Other large, well-filled cells, the cystidia, connect one gill with another. They originate, as do the hymenial cells, in the trama, but these become several times larger than the surrounding cells and

finally protrude into the opposite gill. They are club-shaped, binucleate, and contain many vacuoles. Cystidia have been the cause for much speculation, and a wide variety of functions has been given them (fig. 3).

Two nuclei are always present at the base of the young basidial cell (fig. 4). The remainder of the cell contains cytoplasm and large vacuoles which, at this time, are present in the center and near the apex. These two primary nuclei move to the center of the cell and fuse. At first the two nucleoli are separate in the single nucleus, but soon merge into one (figs. 5–7). The resulting nucleus immediately swells and synapsis begins within (fig. 8).

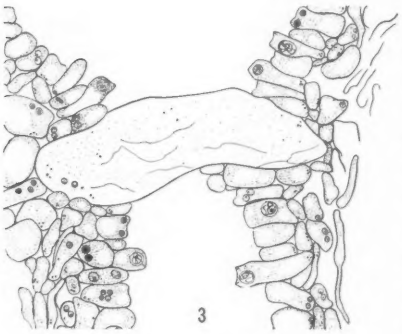
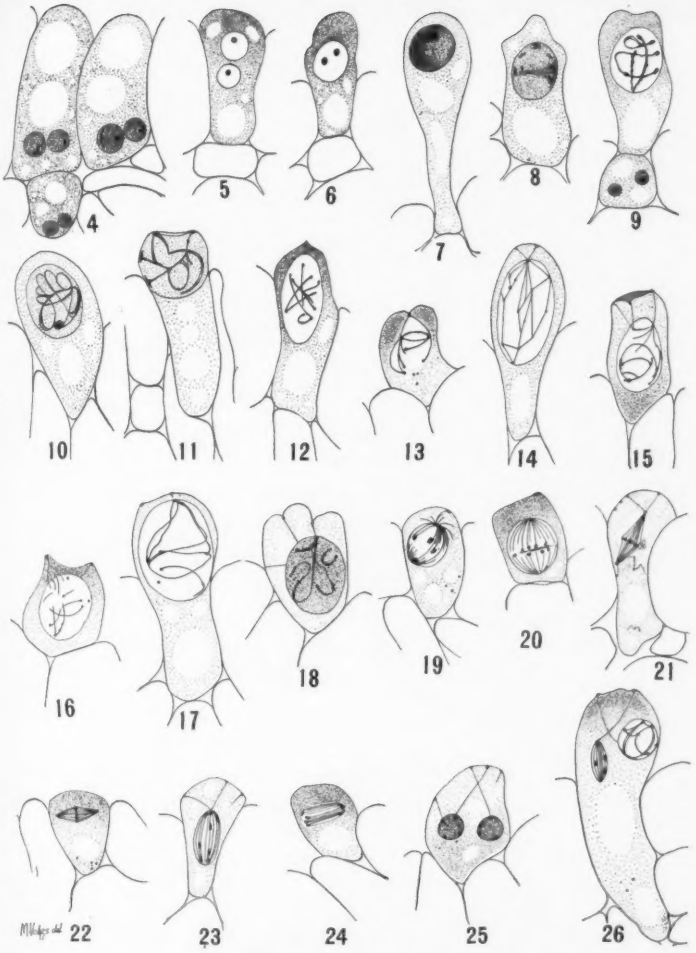


FIG. 3.—Hymenial layer showing cysidia

During the prophases the chromosomes appear to be arranged in a more or less continuous spireme. At times this appears to be rather irregular (fig. 9), and the nucleolus remains distinct (fig. 10) but soon disappears. The spireme becomes threadlike and shows longitudinal splitting (fig. 11). While the prophases are in progress the nucleus is gradually migrating upward, until it reaches the apex of the basidium, where it assumes large proportions, at times occupying the entire space and touching the basidial wall (fig. 11). It does not seem to stay in contact with the wall for any length of time, for it has always withdrawn by the time the spireme within it segments (fig. 12). This slight contact, however, permits the nuclear membrane to leave tiny hyaline bodies attached in four places to the interior margin on the wall above. These hyaline bodies appear to be homologous with the structures which MAIRE (**22**) and LEVINE (**17–19**) call centrosomes, although they are distinct from those found later at the poles of the spindles. As the nucleus withdraws from the wall it seems to pull out fine fibrillar strands from the four hyaline bodies. These strands remain attached to the nucleus, and



FIGS. 4–26.*—Figs. 4, 5, primary binucleate basidial cells; fig. 6, recent nuclear fusion, two nucleoli still present; fig. 7, same, nucleoli now fused; fig. 8, nucleus enlarged; synapsis beginning; figs. 9, 10, prophase, enlarged nucleus migrating to apex of basidium; fig. 11, nuclear membrane in contact with basidial wall at apex; fig. 12, nucleus moving toward center of cell; figs. 13–17, cytoplasmic strands attached to both hyaline bodies and prophase nucleus; fig. 18, basidial wall pulled in by downward movement of prophase nucleus; hyaline bodies attached firmly to wall and fibers tight; fig. 19, four chromosomes appear to be bivalents; fig. 20, disjunction of chromosomes reduction division; fig. 21, equatorial plate stage; fig. 22, position of spindle lying at different angle from that in fig. 21; fig. 23, anaphase; fig. 24, early telophase; fig. 25, two daughter nuclei; fibrillar strands remain attached, two to each nucleus; fig. 26, second nuclear division.

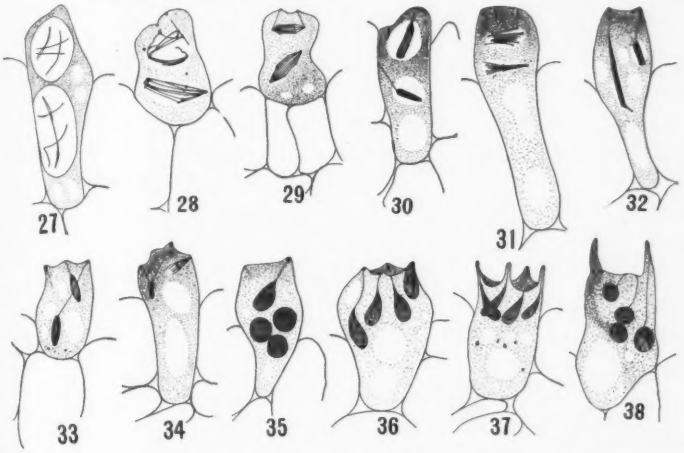
* Figs. 4–48 were made with Zeiss 2 mm. apochromatic objective (N.A. 1.40 and 1.30) combined with Zeiss compensating ocular 15×.

later to the daughter nuclei which result from the division of the parent (figs. 11–18). It is not always possible to show four such fibrillar strands, but four hyaline bodies are certainly laid down by the single nucleus during the contact of membrane and cell wall, the fibers being pulled out by the withdrawal of the nucleus to the center of the basidium. By the time the nucleus has returned to the middle of the cell the spireme within has become short and segmented (figs. 12–16). A spindle is then formed which presents the metaphase, the chromosomes being present at the equator. Four chromosomes are seen which appear to be bivalents (fig. 19). Fig. 20 shows four chromosomes passing to each pole, and fig. 24 represents the telophase in which the chromosomes have reached the poles. Two daughter nuclei are then formed which are about the size of the nuclei in the young basidium. Each of these has cytoplasmic fibers attached to two hyaline bodies at the apex of the basidial wall. These fibrillar threads are not always straight, sometimes appearing to be loose, but more often found crossed or twisted about each other, giving the appearance of branching. They always extend, however, from the hyaline body on the wall to the nuclei below (figs. 25, 26).

The second reduction division immediately takes place (figs. 26, 27). In some instances one nucleus in a basidium will be in prophase while the other has a spindle formed (fig. 26). The nucleus when in prophase is always larger. Usually the divisions of two nuclei in one basidium are simultaneous, the four small nuclei appearing at about the same time (fig. 35). When four nuclei are present in the basidium each nucleus is attached by a fibrillar strand to a hyaline body above. Figs. 29, 32–41 show the influence of the hyaline bodies to both basidial wall and nuclei. Often, before the second division is completed, the basidial wall bulges up in four places, where a hyaline body remains within at the apex of each protrusion. By the time the four nuclei have developed great progress has been made by the sterigmata, which have protruded and the nuclei have begun their upward movement.

The hyaline bodies remain fixed to the apex of the growing spore wall, which enlarges greatly after the entrance of the nucleus. Within the spore cytoplasmic threads seem to be in a variety of positions. No distinct nucleus can be determined, but the hyaline bodies can

usually be discerned at the apex. This body, at one time so closely associated with the fusion nucleus, has remained at the apex of the basidial wall during two mitoses, goes upward within the peak of the sterigmata, and is at the apex of the young spore (figs. 11–15, 28–48). Since no definite orientation of spindles is present, a spindle will sometimes be in such a position as to block a nucleus from entering



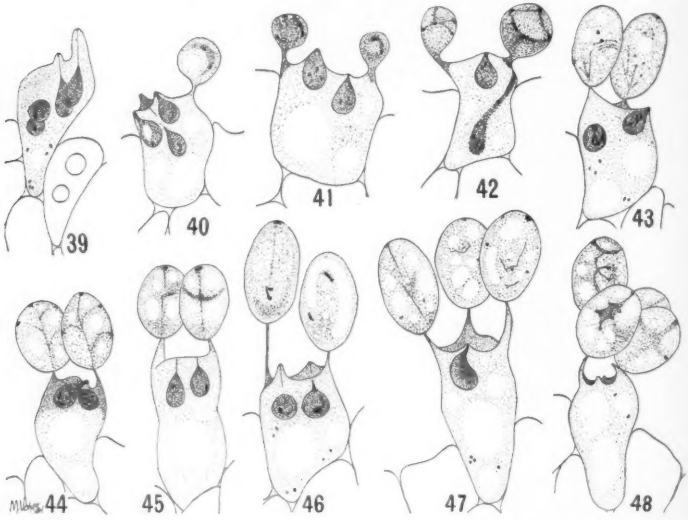
FIGS. 27–38.—Fig. 27, prophase of second division; fig. 28, spindle formation and spindle in anaphase; fig. 29, anaphase; spindle showing four chromosomes and attachment to hyaline bodies at both poles; figs. 30–32, spindles in various stages of division; fig. 33, early telophase; cell wall projects decidedly where hyaline bodies are attached; fig. 34, spindles in early telophase and equatorial plate stages, latter attached directly to hyaline body; fig. 35, four nuclei formed; fig. 36, four nuclei, each attached to a strand, migrating toward projection where hyaline body is attached; figs. 37, 38, migration of nuclei.

a sterigma. These variations probably account for the differences in the maturation of spores.

In the young basidia the two nuclei are at the base of the cell, and large vacuoles are present in the center and near the apex. After fusion and migration of the nucleus to the apex, the vacuoles change their position to the base. The cytoplasmic content of the cell is not dense. The placement of the hyaline body on the wall is important, for where it is located protrusion occurs, although at no time does

the wall appear to be thinner at that place. After the spores are formed the basidium continues to contain cytoplasm and large vacuoles. The basidial cell remains rigid and does not break down. Eight chromosomes are thought to be present in the fusion nucleus, and reduction apparently takes place in the first division.

WAGER (31), LEVINE (17–19), and KUHNER (15) agreed that the single nucleus moved to the apex of the basidium. They also ob-



FIGS. 39–48.—Fig. 39, nuclei in various stages of migration; fig. 40, single spore in early stage; three nuclei still in basidial cell but nearing sterigmata; fig. 41, two young spores formed and two nuclei migrating; fig. 42, spore membrane enlarged after entrance of nucleus; figs. 43–46, two spores reaching maturity; two nuclei still within basidium; fig. 47, three spores formed; delayed nucleus migrating; fig. 48, four spores formed; basidial cell now devoid of nuclei.

served "centrosomes" and fibrillar threads. Fibrillar threads, MAIRE (22) thought, contracted and brought the nucleus up into the sterigma. LEVINE (19) observed these fibers, but not until four daughter nuclei were produced, after which the fibrillar strands apparently pulled the nuclei up into the spores. This hardly seems necessary, for the "centrosome" which is attached to the apex of the sterigma moves up with the growth of the spore (which is not a spore initial

but an outgrowth or protrusion of the spore membrane), and this fiber is certainly not any longer, if it is as long, as the height of the sterigma and spore combined; and the nuclear content undoubtedly is coherent. The nucleus then merely passes along with the elongation of the sterigma and spore and appears to be held by the fibrillar strand.

WAGER'S phenomena regarding the homologous massing of nuclei at the base of the basidium was not apparent in this study, and was probably caused during fixation. It is quite possible that astral rays occur, as they do in some fungi, but it is also possible that what were interpreted as astral rays may have been cytoplasmic strands which broke during fixation and hung down into the cytoplasmic content from the poles of the dividing nuclei. With further study it is hoped to determine this point.

Summary

1. After fusion of the two nuclei in the basidial cell of *Coprinus atramentarius*, synapsis takes place in the single nucleus. This nucleus then migrates to the apex of the cell and its membrane comes in contact with the cell wall, where four hyaline bodies become attached. With the withdrawal of the nucleus to the center of the cell cytoplasmic threads are drawn out. These connect the hyaline bodies left on the wall to the nucleus.

2. The centrosomes of the spindles are not interfered with in the formation of the four hyaline bodies which remain on the basidial wall. After the first division, threads from four hyaline bodies are attached to the two nuclei; after the second division, threads from four hyaline bodies are attached to as many nuclei.

3. The long axis of the spindle has no definite orientation in either division.

4. The hyaline bodies remain attached to the apex of the cell wall from the time the fusion nucleus comes in contact with it until after formation of the spores. These bodies react on both the wall and the nucleus in such a way that the wall pushes up at their points of contact and sterigmata form. The nucleus then follows, and after it has entered the spore the wall greatly enlarges and assumes mature proportions.

5. After the hyaline bodies become attached they remain at all times at the apex. After a sterigma forms a hyaline body is at the tip as well as after the spore is formed.

6. There are eight chromosomes in the fusion nucleus. Reduction takes place in the first division.

I wish to express my thanks to Professor C. J. CHAMBERLAIN, who proposed this work; and to acknowledge my indebtedness to Dr. S. YAMANOUCHI for making the slides and microscopically verifying figures.

10182 GRATIOT AVE.
DETROIT, MICH.

[*Accepted for publication November 2, 1929; delayed through author's revision*]

LITERATURE CITED

1. ATKINSON, G. F., Development of some species of *Hypholoma*. Ann. Mycol. **4**:387–394. 1906.
2. ———, The development of *Agaricus campestris*. BOT. GAZ. **42**:241–264. 1906.
3. ———, The development of *Agaricus arvensis* and *A. comtulus*. Amer. Jour. Bot. **1**:3–22. 1914.
4. DANGEARD, P. A., Recherches histologiques sur les champignons. Le Botaniste **3**:63–143. 1891.
5. ———, Recherches sur la reproduction sexuelle des champignons. Le Botaniste **3**:221–286. 1891.
6. ———, Mémoire sur la reproduction sexuelle des Basidiomycètes. Le Botaniste **4**:119–181. 1895.
7. ———, Nouvelles considérations sur la reproduction sexuelle des champignons supérieurs. Le Botaniste **9**:35–46. 1906.
8. DE BARY, L., Comparative morphology and biology of fungi. Eng. ed. 1918.
9. FITZPATRICK, H. M., The cytology of *Eocronartium muscicola*. Amer. Jour. Bot. **5**:397–499. 1918.
10. FRIES, R. E., Zur Kenntnis der Cytology von *Hygrophorus conicus*. Svensk. Bot. Tidskr. **5**:241–251. 1911.
11. GILBERT, E. M., Studies on the Tremellineae of Wisconsin. Wis. Acad. Sci. Lett. Trans. **16**:1137–1170. 1910.
12. ———, Nuclear phenomena in *Basidia*. Science N. S. **33**:264. 1911.
13. HARPER R. A., Binucleated cells in certain Hymenomycetes. BOT. GAZ. **22**:1–23. 1902.
14. HOFFMAN, H., Die Pollinarien und Spermatien von *Agaricus*. Bot. Zeit. **14**:137–148. 1856.

15. KUHNER, R., Contribution à l'étude des Hymenomycètes, et spécialement des Agaricaces. Le Botaniste **17**:1–215. 1926.
16. ———, Etude Cytologique de l'hymenium de *Mycena galericulata* Scop. Le Botaniste **18**:169–176. 1927.
17. LEVINE, N., Studies in the cytology of the Hymenomycetes, especially the *Boleti*. Bull. Torr. Bot. Club **40**:137–161. 1913.
18. ———, Origin and development of the lamellae in *Agaricus campestris* and certain spp. of *Coprinus*. Amer. Jour. Bot. **9**:509–533. 1922.
19. ———, Origin and development of the lamellae in *Coprinus micaceus*. Amer. Jour. Bot. **1**:343–356. 1914.
20. MAIRE, R., Sur la cytologie des Hymenomycètes. Compt. Rend. Acad. Sci. **131**:121–124. 1900.
21. ———, Sur la cytologie des Gastromycètes. Compt. Rend. Acad. Sci. **131**: 1246–1248. 1900.
22. ———, Recherches cytologiques et taxonomiques sur les Basidiomycètes. Thèses, Faculté Sciences, Paris. 1902.
23. NICHOLS, S. P., The nature and origin of the binucleated cells in some Basidiomycetes. Trans. Wis. Acad. Arts Lett. **15**:35–70. 1904.
24. PETRI, L., La Formazione delle spore nell'Hydnangium carneum. Wallr. Nuov. Giorn. Bot. Ital. II. **9**:499–514. 1902.
25. ROSEN, F., Studien über die Kerne und die Membranbildung bei Myxomyceten und Pilzen. Cohn. Beitr. Biol. Pfl. **6**:237–264. 1893.
26. ROSENVINGE, L. K., Sur les noyaux des Hymenomycètes. Ann. Sci. Nat. Bot. VII. **3**:73–93. 1886.
27. RUHLAND, W., Zur Kenntniss der Intracellularen Karyogamie bei den Basidiomyceten. Bot. Zeit. **59**:187–206. 1901.
28. STRASBURGER, E., Das Botanische Praktitum. 1884–87.
29. VAN BAMBEKE, C., Sur l'évolution nucléare et la sporulation chez Hydnangium carneum. Wallr. Mém. Acad. Roy. Sci. Belg. **54**:1–44. 1903.
30. WAGER, H., On nuclei of the Hymenomycetes. Ann. Botany **6**:146–148. 1892.
31. ———, On nuclear division in the Hymenomycetes. Ann. Botany **7**:498–514. 1893.
32. ———, On presence of centrospheres in fungi. Ann. Botany **8**:321–334. 1894.
33. ———, Sexuality in fungi. Ann. Botany **13**:575–597. 1899.

PHOTOTROPIC "INDIFFERENCE" AND THE LIGHT-SENSITIVE SYSTEM OF PHYCOMYCES[1]

E. S. CASTLE

(WITH TWO FIGURES)

I

A state of phototropic "indifference" is said to exist in the light-sensitive sporangiophore of *Phycomyces* when a phototropic growth response fails to follow exposure of the sporangiophore to lateral illumination from one source. The phototropic response is termed positive when bending is toward the source of light; negative when bending is away from it. The existence of these two types of bending led earlier workers to regard the state of indifference as a resultant of two opposed phototropic reactions (OLTMANNS **6**, BLAAUW **1**). It has been shown (CASTLE **4**, **5**) that there is in the sporangiophore of *Phycomyces* one basic light-sensitive system which, according to the conditions of illumination and observation, determines two types of photic response: (1) a direct growth response, manifested as a brief acceleration in the rate of elongation of the sporangiophore; and (2) a phototropic response, or bending of the sort already mentioned. Evidence is here submitted to show that phototropic indifference in *Phycomyces* is due to equal stimulation and response on both sides of the sporangiophore, based on photic excitation occurring within one photosensitive system. An account of the mechanism of the reversal of phototropism, leading to negative bending, will be given elsewhere.

"Indifference" has been studied previously under conditions of continuous unilateral illumination (**6**). This method has the disadvantage that the sensitivity to light of sporangiophores which have been allowed to become dark adapted is known to change rapidly during illumination (TOLLENAAR and BLAAUW **7**, CASTLE **3**). Furthermore, changes in the pigmentary content of the sporangiophore may well be induced by long continued illumination.

[1] From the Laboratory of General Physiology, Harvard University, Cambridge.

In the following experiments indifference was studied following exposure to relatively brief flashes of light. Under these circumstances the sensitivity of the sporangiophore cannot change appreciably, the exposure times being as brief as one second or less. With sporangiophores of high sensitivity, illuminated from one side by light of 171 ft.-candles, a state of phototropic indifference was found over a range of exposure extending from continuous illumination down to a duration of exposure of about 0.6 second. When the exposure is further shortened, positive phototropic bending occurs.

If a sporangiophore which exhibits phototropic indifference is exposed to lateral illumination under the same circumstances, it is found that a distinct direct growth response takes place. Stimulation followed by phototropic indifference is therefore not characterized by the absence of photic excitation, but by the absence of a differential growth response.

II

The apparatus used in studying the occurrence of indifference permitted optional observation of either the direct growth response or the phototropic response, and has already been described in detail (5). It consisted essentially of a moist chamber with plate glass walls and cover held at constant temperature ($24.5 \pm 0.1°$ C.) in a water thermostat, the whole inclosed by a small darkroom. Observation of a particular sporangiophore in a culture placed in the chamber was made from outside the darkroom by means of a horizontal microscope having an ocular scale. This scale could be rotated, and permitted measurement of either vertical growth or horizontal bending.

The orienting and adapting light, giving an illumination of 86 ft.-candles, was a 100-watt bulb directly above the observation chamber. Sporangiophores were brought to a state of uniform high sensitivity by thorough adaptation to this light, followed by dark adaptation of 30 minutes' duration. Dark adaptation has been shown to take place nearly to completion within this time (3).

The stimulating illumination came from a 1000-watt lamp in a special housing above the observation cell. It was reflected horizontally on to the sporangiophore by means of a 45° mirror, after passing through a heat screen and a calibrated photographic shutter

arranged to grade the duration of the exposure. The intensity of this light at the sporangiophore was 171 ft.-candles.

Readings of the position of the sporangium on the ocular scale were made at 15-second intervals against a faint red observation light. Plottings of the readings were made against time. The moment of response was taken as the first point significantly deviating from the pre-existing direction of growth, and the reaction time (*R.T.*) as the interval between the beginning of stimulation and the moment of response. Pure cultures of *Phycomyces blakesleeanus* (+ strain) were used throughout the experiments.

III

The phototropic response of the sporangiophore of *Phycomyces* has been shown to occur practically simultaneously with the direct growth acceleration (4). In these experiments, therefore, the significant presence or absence of phototropic bending is noted at and near the time when photic response of the sporangiophore is known to take place. Bending occurring much later than this time is not an immediate sequel of the primary photic excitation, and does not indicate the absence of primary "indifference."

It was found that with a duration of exposure greater than 0.6 second to unilateral illumination of 171 ft.-candles, no primary phototropic response of the sporangiophore occurred. A state of phototropic indifference therefore existed. With an exposure of 0.6 second, irregular and occasional bending responses were obtained, as shown in detail in fig. 1. As the exposure time is further shortened, the occurrence of the phototropic response becomes invariable. Its regularity for different brief durations of exposure is made clear by the frequency polygons of fig. 1.

Table I gives the mean reaction times of the phototropic response with varied durations of exposure. These figures are plotted in fig. 2. The significance of the form of the curve, in showing that the velocity of the principal process taking place during the latent period is directly proportional to the amount of preceding photochemical change, has already been discussed (5). It has also been shown that the comparable curve for the direct growth response is superimposable upon the curve of fig. 2. This finding is taken to indicate

that both the direct and the phototropic modes of response are based on the same photosensitive system.

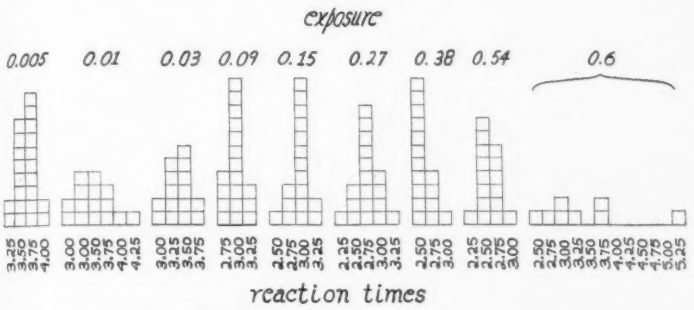


FIG. 1.—Frequency polygons showing actual distributions of individual determinations of phototropic reaction times for different durations of exposure to stimulating illumination (171 ft.-candles); exposures given in seconds above each polygon; reaction time classes indicated on abscissae; each square stands on ordinate scale for one determination of reaction time.

The reaction time for the direct growth response with exposures greater than about 0.6 second is approximately constant. Below that critical duration of exposure the reaction time lengthens progressively as the exposure shortens. Considering the processes involved in the response to light as necessarily a catenary sequence initiated by

TABLE I

MEAN REACTION TIMES OF PHOTOTROPIC GROWTH RESPONSE TO VARIOUS DURATIONS OF EXPOSURE TO UNILATERAL ILLUMINATION OF 171 FT.-CANDLES

EXPOSURE (SECONDS)	MEAN *R.T.* (MINUTES)*	PROBABLE ERROR OF MEAN *R.T.*
0.005	3.63	±0.028
0.01	3.50	0.061
0.03	3.38	0.040
0.09	2.98	0.024
0.15	2.96	0.030
0.27	2.76	0.037
0.38	2.60	0.048
0.54	2.61	0.028

* Each mean *R.T.* represents average of 15–22 determinations on individual sporangiophores (such averaging of photic responses is justifiable in spite of the variability in absolute rate of growth, **2, 3**).

the photochemical action of light, it is clear that with such brief exposures not enough photochemical change takes place to cause the subsequent latent period processes to proceed at a maximal rate. For the direct growth response, then, a duration of exposure of 0.6 second to light of 171 ft.-candles is critical: above that value the latent period processes involved in the response proceed at a con-

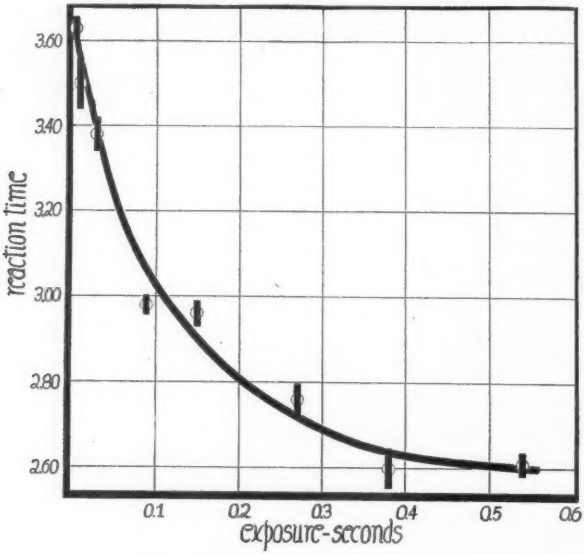


FIG. 2.—Mean reaction times (in minutes) of phototropic response plotted against duration of exposure to stimulating illumination; height of vertical bar through each symbol represents twice probable error of mean; curve is arbitrary, to show regular decrease in reaction time with increasing duration of exposure.

stant maximal rate; below that value such secondary changes are retarded in inverse proportion to the duration of the exposure (4).

For the phototropic response, also, a duration of exposure of 0.6 second is critical, in that it is the dividing line between the presence and absence of phototropic indifference, an inverse corollary of bending. For briefer durations of exposure the photochemical action of the light is evidently submaximal, at least on one side of the spor-

angiophore. Unequal action of light on the two sides must initiate secondary changes which proceed at different rates, and specifically the latent period processes on one side will advance compared with those on the other.

The observed result, bending, is thus a differential growth response, produced in this instance because the amount of light admitted to the sporangiophore was reduced until the response on one side was retarded relative to that on the other. A slight relative retardation might be expected to lead to a brief inflection of the sporangiophore, followed by an early abolition of the differential effect. Exactly such brief bendings are found with durations of exposure close below 0.6 second.

Summary

1. Sporangiophores of *Phycomyces* of high sensitivity to light, stimulated by exposure to unilateral illumination of sufficiently high intensity, fail to give the usual phototropic response. This condition is known as phototropic "indifference."

2. Sporangiophores which exhibit such indifference are nevertheless shown to give a distinct direct growth response as a consequence of the same unilateral illumination. The state of indifference is therefore not characterized by the absence of photic excitation, but by the failure of the light to evoke a differential acceleration of growth on the two sides of the sporangiophore.

3. Stimulation of sensitive "indifferent" sporangiophores by flashes of light of progressively reduced duration of exposure leads to the discovery of a critical duration below which indifference is abolished and phototropic bending occurs.

4. The critical duration of exposure to light for the appearance of the phototropic response corresponds to the critical duration of exposure for minimum reaction time in the direct growth response.

5. Phototropic bending therefore appears when the action of light on one side of the sporangiophore is submaximal. Conversely, indifference is due to equal and maximal photochemical action on both sides.

Part of the expense incidental to this investigation was defrayed by a grant from the Milton Fund of Harvard University.

UNIVERSITY MUSEUM
CAMBRIDGE, MASS.

[*Accepted for publication February 26, 1930*]

LITERATURE CITED

1. BLAAUW, A. H., Die Perzeption des Lichtes. Rec. Trav. Bot. Néerl. **5**:209–372. 1909.
2. CASTLE, E. S., Temperature characteristics for the growth of the sporangiophores of *Phycomyces*. Jour. Gen. Physiol. **11**:407–413. 1927–28.
3. ———, Dark adaptation and the light-growth response of *Phycomyces*. Jour. Gen. Physiol. **12**:391–400. 1928–29.
4. ———, The light-sensitive system as the basis of the photic responses of *Phycomyces*. Proc. Nat. Acad. Sci. **16**:1–6. 1930.
5. ———, Phototropism and the light-sensitive system of *Phycomyces*. Jour. Gen. Physiol. **13**:421–435. 1929–30.
6. OLTMANNS, F., Über positiven und negativen Heliotropismus. Flora **83**:1–32. 1897.
7. TOLLENAAR, D., and BLAAUW, A. H., Light and dark adaptation of a plant cell. Proc. Acad. Sci. Amsterdam **24**:15–32. 1921.

SOLUBILITY CHANGES OF INORGANIC CONSTITUENTS IN CITRUS CUTTINGS[1]

F. F. Halma and A. R. C. Haas

(with two figures)

Introduction

In the propagation of citrus trees by cuttings, it has been found that the presence of leaves on the cuttings is essential to successful propagation in some species.[2] This is clearly evident from the fact that detached leaves root readily, while stems without leaves do not ordinarily respond in this way. With hardwood cuttings the stem contains the carbohydrates needed for root development; with citrus cuttings, on the other hand, the carbohydrates are supplied by the leaves, either from that stored up or that currently manufactured. Although considerable attention has been given to the importance of carbohydrate changes during the rooting process of cuttings, yet to the writers' knowledge no data are available as to whether there are any concurrent changes in the solubility of inorganic constituents. It was thought desirable, therefore, to determine what solubility changes, if any, take place in the inorganic constitution.

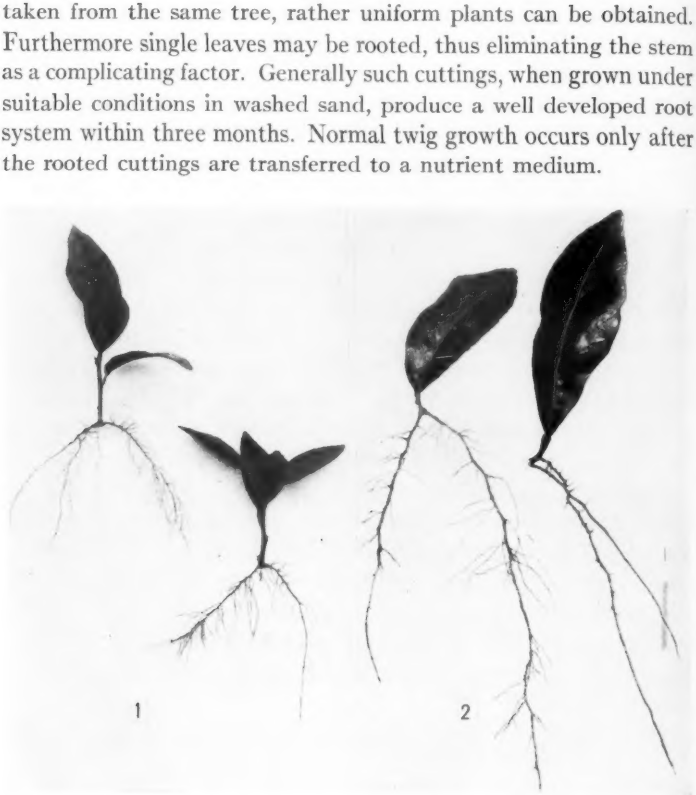
This investigation was purposely confined to the study of solubility changes, rather than to that of the actual amount of translocation, for the reason that in the latter case it would be extremely difficult to determine the amounts involved in material of this kind. With older stem cuttings, having a correspondingly larger root system, this might be possible were it not for the fact that the continuous development of vegetative growth introduces a new factor.

Citrus cuttings, for several reasons, are well adapted to a study of solubility changes of inorganic constituents. Cuttings can be grown successfully at any time of the year, and if of similar age and

[1] Paper no. 219, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Halma, F. F., Propagating citrus by cuttings. Calif. Citrograph 11:225. 1926.

taken from the same tree, rather uniform plants can be obtained. Furthermore single leaves may be rooted, thus eliminating the stem as a complicating factor. Generally such cuttings, when grown under suitable conditions in washed sand, produce a well developed root system within three months. Normal twig growth occurs only after the rooted cuttings are transferred to a nutrient medium.



FIGS. 1, 2.—Fig. 1, rooted stem cutting of Eureka lemon and Navel orange; fig. 2, rooted leaf cutting of Eureka lemon and Navel orange.

Cuttings in sand

Eureka lemon (a variety of *Citrus limonia* Osbeck) and Navel orange (a variety of *C. sinensis* Osbeck) constituted the material for this study. One hundred stem cuttings (fig. 1) and 100 leaf cuttings (fig. 2) of each species were made. Three healthy mature leaves were left attached on each stem cutting. Fifty of each lot were planted in washed sand in a propagation frame and the other fifty were prepared for analysis in the following manner. In the stem cuttings the

apical, middle, and basal leaves, as well as the stems, were analyzed separately. Similarly the 50 leaf cuttings constituted a separate sample. The cuttings rooted satisfactorily in the propagation frame, and after three months they were taken out and prepared for analysis. Unpublished data by HALMA show that there is a high positive correlation between total leaf area or total green weight of leaves and total amount of roots produced, hence it is obvious that the single leaf cuttings produced less roots than the 3-leaved stem cuttings.

TABLE I

SOLUBLE CALCIUM, POTASSIUM, AND MAGNESIUM AS PERCENTAGES OF WATER-SOLUBLE ASH IN THE DRY MATTER

MATERIAL	CALCIUM		POTASSIUM		MAGNESIUM	
	Control	Rooted	Control	Rooted	Control	Rooted
50 Eureka lemon stem cuttings:						
Apical leaves	25.84	26.38	14.55	9.02	8.24	7.95
Middle leaves	24.82	25.95	13.91	11.15	7.75	8.20
Basal leaves	23.96	24.71	13.68	10.94	8.05	7.80
Stems	23.60	14.77	20.95	18.39	10.90	7.52
50 Eureka lemon leaf cuttings	25.87	23.75	12.14	12.05	5.18	6.08
50 Navel orange stem cuttings:						
Apical leaves	28.39	28.64	14.56	12.52	4.74	4.27
Middle leaves	27.28	29.11	15.06	13.79	4.11	4.78
Basal leaves	27.48	28.19	16.33	14.45	4.45	4.67
Stems	21.67	17.73	20.19	21.49	7.22	7.90
50 Navel orange leaf cuttings	29.12	26.63	14.02	13.08	4.44	4.86

Table I gives the soluble calcium, potassium, and magnesium as a percentage of the water-soluble ash in the dry matter. The greatest decrease in calcium occurred in the stem and in the leaf cuttings of the rooted material. The three leaves of the rooted twig cuttings, on the other hand, showed a slight but consistent increase. It would appear at first that the decrease of calcium in the stem can be accounted for by the increase of calcium in the three leaves, and that perhaps no calcium was needed for root development. However, the rooted leaf cuttings where no stem is present also showed a decrease, hence it seems that calcium is translocated during the rooting process.

A general reduction in the percentage of potassium occurred in all of the rooted material, with the exception of the Navel orange stem,

where there was a slight increase. This would also indicate, therefore, that potassium is used in the rooting process. The differences in the percentages of magnesium in control and rooted plants were not consistent. Analytical results obtained for the rootlets were found to be unreliable, owing to adhering sand particles and to the leaching out of soluble salts by washing.

Culture solution studies with rooted lemon leaf cuttings

Mature Eureka lemon leaf cuttings were rooted in washed sand in the propagation frame. Twenty-five similar leaf cuttings were prepared for analysis. After three months the rooted leaves were carefully lifted out of the sand and 25 of these were likewise prepared for analysis. Twenty-five of the rooted leaf cuttings were placed in each of five culture solutions: Hoagland's solution,[3] to which was added: (A) nothing (control); (B) calcium 159 p.p.m., nitrate 493 p.p.m.; (C) calcium 318 p.p.m., nitrate 986 p.p.m.; (D) calcium 477 p.p.m., nitrate 1479 p.p.m.; (E) calcium 636 p.p.m., nitrate 1972 p.p.m. The plants remained in these solutions for three months and then were taken out and prepared for analysis.

It may be mentioned that the leaf cuttings, after having remained in the culture solutions for this period, were curled and showed the prominent veins characteristic of old lemon leaves in the field prior to abscission. This premature aging of leaf cuttings has not been observed in stem cuttings.

It will be seen from table II that the percentage of soluble calcium in the leaves became greater with increasing concentrations of calcium in the culture solution. During the rooting process the percentage was reduced from 16.91 to 15.52. This decrease in the soluble calcium continued in solutions A, B, and C, notwithstanding the fact that the solutions were well supplied with calcium. The soluble calcium of the leaves which grew in solutions D and E showed values that may be considered comparable with those of the unrooted leaves.

[3] Composition expressed as parts per million:

Na	K	Ca	Mg	Fe	Mn	NO_3	Cl	SO_4	PO_4	Total
7	185	159	54	1	0.1	718	10	216	105	1455.1

To this solution traces of elements not ordinarily used in culture solutions were also added.

There was a slight decrease in soluble potassium during the rooting process. After three months in the culture solutions there was an enormous increase, however, the highest value being found in the leaves which grew in solution A and the lowest in those which grew in solution E. The gradient here is reversed as compared with that for calcium. The values obtained for magnesium do not show any appreciable changes.

From the data presented, it would seem that changes in the solubility of calcium and potassium in citrus leaf or stem cuttings occur

TABLE II

SOLUBLE CALCIUM, POTASSIUM, AND MAGNESIUM AS PERCENTAGES OF WATER-SOLUBLE ASH IN THE DRY MATTER

EUREKA LEMON LEAF CUTTINGS	CALCIUM	POTASSIUM	MAGNESIUM
Not rooted	16.91	25.83	5.18
Rooted (not placed in culture solution)	15.52	25.64	6.08
Placed in solutions:			
A	12.41	59.45	6.36
B	13.01	58.64	5.73
C	14.73	55.79	5.65
D	17.38	48.17	5.73
E	18.34	44.05	6.15

during the rooting process. This, together with the enormous increase in soluble potassium when the rooted leaf cuttings were given nutrients, throws some light on the great changes in solubility that may occur under various conditions of growth. The premature aging of leaf cuttings when in culture solutions may be due to an unbalanced condition, brought about by continuous absorption of soluble materials by the rootlets and the absence of an outlet for vegetative growth.

Summary

1. Stem cuttings provided with leaves and single leaf cuttings of Eureka lemon (a variety of *Citrus limonia* Osbeck) and Navel orange (a variety of *C. sinensis* Osbeck) constituted the material for this study. During the rooting process changes occurred in the percentages of soluble calcium and potassium of the water-soluble ash in the dry matter. In the case of stem cuttings, the greatest reduction in soluble calcium was found to occur in the stem, the leaves actually

showing a slight but consistent increase. In leaf cuttings where no stem was present there was also a loss of soluble calcium. In the case of potassium the main loss was sustained by the leaves of the stem cuttings, and a similar reduction also occurred in the leaf cuttings.

2. When rooted lemon leaf cuttings were grown in culture solutions of different concentrations of calcium, it was found that while the soluble calcium content of the leaves did not change to any great extent, the soluble potassium content increased enormously.

3. The solubility of magnesium in the leaves was not affected, either during the rooting process or during subsequent growth in culture solutions.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

[*Accepted for publication April 29, 1930*]

BRIEFER ARTICLES

CELL WALL OF VAUCHERIA GEMINATA

During a recent investigation of the methods of healing and wall formation in certain algae and water molds, it was found necessary to study the composition and structure of the wall of *Vaucheria geminata*. In this connection reference was made to a paper by WURDACK.[1] By means of examination in polarized light, and by checking the color reactions, hydrolysis, and the solubility of the membranes, Miss WURDACK found that:

> the cell wall of *V. geminata* is composed of two layers . . . . pectose and cellulose, the former being on the outside . . . . before treating with the pectose solvents the inner layer reacts negatively to the hydrocellulose reaction. . . . . After removing the outer layer of pectic compounds, the inner layer of the cell wall reacts readily to the cellulose reagents, giving a positive test to the hydrocellulose reaction. . . . . This outer layer is very difficultly permeable to cellulose reagents, particularly those used in the hydrocellulose reactions.

After cutting a filament of *V. geminata* into small sections, the protoplast flows outward, leaving the wall as a hollow cylinder. Through this cylinder the cellulose reagents (iodine potassium iodide and 70 per cent sulphuric acid) may diffuse, and, one must assume, come in contact with the supposed inner cellulose layer. Although innumerable filaments were cut, freed of their protoplasm, and treated with iodine potassium iodide and sulphuric acid, at no time did the inner layer of the cell wall react positively to the hydrocellulose reaction. This would seem to indicate that the inner layer is an extremely thin one, composed either of pectose substances impermeable to the cellulose reagents used or of cellulose heavily impregnated with pectose, which, however, fails to mask the polarized light reaction of the cellulose present.

A cut end of a filament of *V. geminata*, when treated with iodine potassium iodide and 70 per cent sulphuric acid, shows a slight blue color, indicating that there the reagents are in contact with a true cellulose layer.

These facts suggest that the cell wall of this alga is composed of three layers: an outer layer of pectic compounds, a middle layer of cellulose, and an extremely thin inner one either of pectose substances or of cellulose heavily impregnated with pectose.—LADLEY HUSTED, *Oberlin College, Oberlin, Ohio.*

[*Accepted for publication April 17, 1930*]

[1] WURDACK, M. E., Chemical composition of the walls of certain algae. Ohio Jour. Sci. 23:181–191. 1923.

CURRENT LITERATURE

BOOK REVIEWS

The Strasburger text

In the early nineties STRASBURGER planned a comprehensive textbook of botany, and he himself wrote the introduction and morphology, including cytology. He associated with himself FRITZ NOLL, who wrote the physiology; HEINRICH SCHENCK, who wrote the cryptogams; and A. F. W. SCHIMPER, who wrote the phanerogams. SCHIMPER, best known for his extensive travels and his work on plant geography, died, and from the sixth edition up to the present time GEORGE KARSTEN has written the phanerogams. NOLL was the next loss, and from the tenth edition, in 1904, up to the sixteenth, in 1923, LUDWIG JOST wrote the physiology. The most severe loss of all came with the death of STRASBURGER in 1912. From the twelfth edition up to the present time the morphology has been written by HANS FITTING, who succeeded STRASBURGER in the Bonn laboratory. SCHENCK's death in 1927 removed the last of the founders of the famous textbook, and his place has been taken by RICHARD HARDER. JOST resigned after the sixteenth edition (in 1923), and HERMANN SIERP wrote the physiology for the seventeenth edition, which appeared in 1928.

The first English edition, translated by H. C. PORTER, of the University of Pennsylvania, appeared in 1898, followed by editions in 1903, 1908, and 1912, all based upon PORTER's translation; but in the present edition,[1] the sixth, which was revised with the seventeenth German edition by Dr. W. H. LANG of the University of Manchester, the changing authorship of the German editions necessitated such extensive revision that PORTER's name is omitted from the title page, and his connection with the English version is described in the introduction. Like the previous editions, the present one is a free translation into idiomatic English, so that only occasionally some peculiar use of a word or some word arrangement makes us realize that our vernacular was not the original language of the text.

It is gratifying to note the increasing recognition given to American work, both in the text and in the illustrations.

Alternation of generations, the cytological basis for which was so well presented by STRASBURGER, is illustrated with comprehensive diagrams covering

[1] FITTING, H., SCHENCK, H., JOST, L., KARSTEN, G., Transl. by W. H. LANG. STRASBURGER'S Textbook of botany. 1921. 6th English ed. 8vo. pp. xi+799. *figs.* 833. Macmillan & Co. 1930.

the subject from the algae to the orchids; but the terms haploid and diploid are unfortunately regarded as synonyms for gametophyte and sporophyte.

Considering the fact that in Germany and England Latin and Greek are still important features of a university curriculum, and that even students specializing in science are likely to know something of these languages, it seems surprising that *macro* is used throughout instead of the more accurate *mega*. Macro means long while mega means large, and is the equivalent of the Latin *magnus*. Macro was doubtless used at first because it sounds as if it were the opposite of micro. Many American texts used the more accurate terms, megaspore, megasporangium, megasporophyll, etc.

The STRASBURGER text won great popularity from the first, partly because it was so authoritative, and partly because it had a unity which is likely to be lacking in a book with several authors. NOLL, SCHIMPER, SCHENCK, and also KARSTEN, were students and friends of STRASBURGER, closely associated with him in the Bonn laboratory, so that the constant contact and discussion prevented repetitions and overlapping which might otherwise have occurred. As one editor has followed another there has been no change in the general plan of the book; the changes for the most part having been such as the progress of the science has demanded. The illustrations have remained about the same. Scarcely any of the figures of the original work have been removed, but new figures have been added until the latest edition, from which the present translation was made, has 833 excellent illustrations. Many of the colored figures illustrate plants which are used for drugs. The official plants are those of the British Pharmacopoeia, instead of those official in Germany, Switzerland, and Austria.

The literature cited has increased from 20 pages in the fifth English edition to 30 pages in the sixth. A study of this list is interesting. In the morphological section, somewhat more recognition is given to American work than in previous editions; in physiology, American work is scarcely recognized at all; but in the treatment of bryophytes and pteridophytes, works in English receive more attention. On the other hand, KARSTEN'S list is broadly international in scope, and his treatment of the subject shows the same breadth and grasp of botanical problems. In this edition JOST has been succeeded by SIERP, and SCHENCK'S section has been written by RICHARD HARDER. While these two newcomers have made some changes, these do not change the general treatment of the subject. It is to the credit of the various authors of succeeding editions that they have preserved the original plan and unity of the book by presenting the fundamentals of botany, which do not change much with fluctuating theories.

Even where American texts are used in college classes, the STRASBURGER text should be of interest, because it puts the student into contact with German ideas and ideals of botanical science, and a comparison of the numerous editions shows the evolution of botany during the past 25 years, at least as far as it is taught in Germany.—C. J. CHAMBERLAIN.

Methods in plant ecology

A recent number of ABDERHALDEN'S[2] great series on methods of investigation in science is devoted to phases of ecology. In the first portion BEGER[3] discusses the methods in use by the Zurich-Montpellier school of ecology for the analysis of the structure of the plant association. In addition to the descriptions of the methods employed, numerous examples of the application of the methods are given.

The larger portion of the number is devoted to methods employed in the study of plant succession. In it LÜDI[4] sketches the history of the development of the modern concept of plant succession, giving proper emphasis to the part played by the American school of ecology, represented by COWLES, CLEMENTS, and their followers. Such quantitative methods as quadrat studies are described and illustrated by examples from both America and Europe. The advantages of permanent and other quadrats are discussed and exemplified. The methods by which postglacial vegetation has been investigated by means of the fossil pollen from peat bogs are fully described and illustrated by the reproduction of many charts, graphs, and pollen diagrams.

The two papers combine to produce a volume that will be indispensable to all workers in plant ecology.—G. D. FULLER.

Life history of flowering plants of central Europe

A useful and rather detailed handbook,[5] giving the ecological life history of the flowering plants of Germany, Austria, and Switzerland, is in process of publication. To date 36 serial numbers (Lieferungen) have appeared, and a number of additional sections are to be published. The earlier numbers cover the gymnosperms, monocotyledons, and several families of the dicotyledons. The numbers here reviewed include the Salicaceae, Myricaceae, Oxalidaceae, Euphorbiaceae, Tiliaceae, and Orchidaceae. Totally or in part the discussion covers the individual species, while the families and genera in general receive only a short treatment, including a bibliography. The most interesting facts about the individual species are given from a biological and morphological point of view, and anatomical details, as species characters, are added when they are of special

---

[2] ABDERHALDEN, EMIL, Handbuch der biologischen Arbeitsmethoden. XI: Chemische, physikalische und physikalisch-chemische Methoden zur Untersuchung des Bodens und der Pflanze. Teil 5, **3**:481–728. Urban & Schwarzenberg. 1930. RM 13.50.

[3] BEGER, HERBERT, Praktische Richtlinien der strukturellen Assoziationsforschung im Sinne der von der Zürich-Montpellier Schule geübten Methode. In ABDERHALDEN'S Handbuch der biologischen Arbeitsmethoden. XI, **5**:481–526. 1930.

[4] LÜDI, WERNER, Die Methoden der Sukzessionsforschung in der Pflanzensoziologie. In ABDERHALDEN'S Handbuch der biologischen Arbeitsmethoden. XI, **5**:527–728. 1930.

[5] Lebensgeschichte der Blütenpflanzen Mitteleuropas. Edited by WANGERIN, W., and SCHRÖTER, C., Lieferung 31–36. Eugen Ulmer, Stuttgart. 1930.

importance. There are ample illustrations, and the handbook should prove of considerable taxonomic value for a student of the central European flora.—A. C. NOÉ.

A chemistry of protoplasm

A new volume has appeared in the series *Protoplasma Monographien*,[6] dealing with the chemical conditions of protoplasm. In this book KIESEL makes a valuable contribution to biochemistry. In the introductory chapter protoplasm in general is discussed; the following chapters consider cytoplasm and its chemical constituents, the nucleus and its constituents, the nucleus during rest and division, the chemical substances found in the nucleus, protoplasm without nucleus, and finally the plasmodia of *Myxomycetes*. A bibliography of 568 titles is included.—A. C. NOÉ.

NOTES FOR STUDENTS

Chlorophyll content and photosynthesis.—There are usually distinguished two internal factors affecting the process of photosynthesis: chlorophyll and the enzymatic or protoplasmatic factors. It must be admitted, however, that there are few definite data as to the relation of these factors to this process. Since the work of WILLSTÄTTER and STOLL, we have had reliable methods of determining the chlorophyll content of plant tissues, but the main difficulty of studying the relation of this factor to photosynthesis has been the impossibility of varying experimentally the amount of chlorophyll present. The usual method has been to use green and yellow varieties of the same species of plant, but the investigators could not be sure that the varieties were the same in all respects except their chlorophyll content. We are indebted for most of our knowledge of these two internal factors to WILLSTÄTTER and STOLL. In the case of chlorophyll, although some of their experiments showed a rough correlation between chlorophyll content and photosynthesis, in general there was no definite correlation. They did much work on the effects of variations in light intensity and temperature on the rate of photosynthesis of green and yellow varieties. They found that in the case of green varieties an increase in temperature caused an increase in photosynthesis, while an increase in light intensity had relatively little effect. The yellow varieties, however, were more affected by light than by temperature. They also found that it took a much higher light intensity to get maximum photosynthesis in the yellow varieties than in the green. The different effects of light and temperature on the green and yellow varieties were regarded by these workers as supporting the viewpoint that there are involved in photosynthesis two stages or series of stages, one photochemical and the other chemical.

EMERSON[7] seems largely to have solved the difficulties in studying the effects of variation in the chlorophyll content on photosynthesis, at least in the case

[6] KIESEL, A., Chemie des Protoplasmas. pp. viii+302. Gebrüder Borntraeger, Berlin. 1930. M. 20.

[7] EMERSON, ROBERT, The relation between the maximum rate of photosynthesis and concentration of chlorophyll. Jour. Gen. Phys. **12**: 609–622. 1929.

of one of the lower plants. He used the alga *Chlorella vulgaris*, and varied the chlorophyll content by growing it in a nutrient solution of varying iron content, and, in order to get healthy cells of normal metabolism in the case of cultures of low iron content, he used a nutrient solution containing about 1.5 per cent glucose. With glucose in the culture solution, it was found that by varying the iron content, cells of nearly the same size and same rate of respiration, but of very different chlorophyll content, could be obtained. With material secured in this way EMERSON found that as the chlorophyll content increased photosynthesis increased proportionally. The curves obtained by plotting the rate of photosynthesis against chlorophyll content were practically straight lines.

By other experiments, EMERSON[8] found that contrary to the results of WILLSTÄTTER and STOLL, the maximum rate of photosynthesis was reached at about the same light intensity no matter what the chlorophyll content. The actual rate of photosynthesis was much higher in the case of the higher chlorophyll content. Also, over this same range of chlorophyll content the process of photosynthesis showed about the same relationship to temperature. The chemical phase of photosynthesis, the part that has been thought of as enzymatic in nature, has been regarded as being readily inhibited by prussic acid. EMERSON, however, found that prussic acid inhibits photosynthesis more in the lower chlorophyll concentration than in the higher. The results of this paper are really not in accordance with the viewpoint that photosynthesis is made up of photochemical and chemical phases. EMERSON suggests that perhaps chlorophyll plays a part, not only in the photochemical phase but also in the chemical, and that photosynthesis may involve an autocatalytic reaction.

If these results are substantiated, it would seem that EMERSON has worked out a method which should enable investigators to study more accurately the relation of chlorophyll content to the process of photosynthesis than has been possible in the past.—S. V. EATON.

---

[8] EMERSON, ROBERT, Photosynthesis as a function of light intensity and of temperature with different concentrations of chlorophyll. Jour. Gen. Phys. **12**: 623–639. 1929.